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14. ABSTRACT
The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved.

15. SUBJECT TERMS
Lifestyle; Cardiovascular Disease (CVD); Prevention; Behavior Modification; Coronary Heart Disease (CHD); Genomics; Metabolic Syndrome; PreDiabetes; Diabetes; Obesity; Stress; Sleep; CVD Risk Reduction

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INTRODUCTION

In 1998, Congress supported the need for basic and clinical research in Coronary Artery and Prostate Disease in order to reduce the incidence of these life-threatening diseases and develop more effective, more specific, and less invasive forms of therapy for patients (Public Law No. 105-262). In FY10, the Integrative Cardiac Health Project (ICHP) was identified as a cardiovascular (CV) research Center of Excellence (COE) by Health Affairs and placed into the Army Program Objective Memorandum (POM). ICHP continues its operation at the Walter Reed National Military Medical Center (WRNMMC) in Bethesda, Maryland.

Heart disease is the most common, costly, and preventable of all health problems and the Military Health System (MHS) has a large number of beneficiaries at risk for CVD. Cardiac related events make up a significant portion of non-battle disease injuries requiring evacuation from Theater jeopardizing operational effectiveness. Service members with multiple combat deployments and Wounded Warriors have increased CVD morbidity/mortality risk (2 and 3.5 fold, respectively).[1,2] Despite optimal medical therapy such as statins, there remains a residual CVD risk of approximately 69%.[3] Existing wellness programs in the Department of Defense and civilian healthcare do not adequately address CVD risk or obstacles related to healthy living which contribute to escalating CVD risk. This large gap in care can only be addressed with innovative, intensive, multi mechanistic approaches to improve CVD outcomes. There is a critical need for personalized CVD risk reduction and actionable empowerment strategies/tools to optimize health and reduce cost.

The ICHP champions the way for optimal CV Health in the MHS by conducting novel research utilizing a personalized medicine (systems biology design) to discover and develop practical, pre-emptive and integrative approaches in order to detect and combat CVD earlier and augment traditional care before it affects the quality of life. This vision is in support of the MHS Strategic Focus and Quadruple Aim on health and wellness and complements the Army Performance Triad.

ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice in an effort to achieve the following research aims:

- Improve Force Health by better understanding the CVD risk susceptibility of military specific populations as well as to understand the individual service member through leading-edge research using novel tools and technologies.
- Investigate and create transformational models of healthcare delivery through personalized CVD prevention tracks as an adjunct to traditional care.
- Refine individualized prevention strategies through statistical data modeling to define the most cost-effective and sustainable approaches in promoting cardiovascular health throughout the military lifecycle.
- Simultaneously, improve understanding of the molecular, physiological, biochemical, immunological and environmental basis of CV health and disease and to use that understanding to develop improved approaches to disease diagnosis, treatment and prevention, in line with NHLBI Strategic Plan 2008.

BODY

During this period of performance, ICHP has introduced its cardiovascular health model, vision, and research and scientific accomplishments to a number of internal and external stakeholders, as well as potential stakeholders for potential synergies. Briefings were provided to the following committees/individuals:

1) The Defense Center of Excellence (DCOE) Oversight Board chaired by Dr. Warren Lockette at the Military Health System (MHS)
2) RADM Elizabeth Niemeyer, DCOE Representative for the Navy
3) LTG Patricia Horoho (Army Surgeon General) at the Performance Triad Workshop
4) WRNMMC Board of Directors and RADM Alton Stock
5) Dr. Robert Jesse, Principle Deputy Undersecretary of VA Health
6) Dr. O'Reilly, Director of Veterans Administration (VA) Research
7) Dr. Allison Haskell, Undersecretary of VA Benefits
8) Dr. Patricia Hayes, VA Women's Health

In addition, Meadville Medical Center in Meadville, Pennsylvania requested to use the ICHP Cardiovascular Disease Prevention Program (CPP) in a demonstration project to be conducted at Meadville and potentially Windber Medical Center. The initiative was under development through a partnership development at HJF, but never fully implemented upon departure of the main collaborator at Meadville.

ICHP underwent an independent scientific review by the American Institute of Biological Sciences (AIBS) in December 2012 at the request of USAMRMC and TATRC. As a result of recommendations given by this review, a Scientific Review Board (SAB) was formed. The board consists of experts in cardiovascular diseases, a biostatistician, a cardiologist, an endocrinologist, and an expert in design of clinical trials. The ICHP SAB met in April 2013, December 2013 and April 2016. Current research initiatives were reviewed and new directions of research and translational opportunities examined. The ICHP SAB findings validated ICHP's future scientific roadmap on both the clinical and molecular level.

The following significant ICHP achievements should be highlighted in this final report. An ICHP manuscript[4] was included as evidence to support the new Clinical Guideline change to include family history as a significant CVD risk factor by the American Heart Association and American College of Cardiology Expert Panel 2013 for New Guidelines in CVD Risk assessment.[5] This evidence also impacted the 2014 Mayo Clinic CVD Risk Reduction Guidelines. ICHP's life management model has been translated into practice complementing the US Army Surgeon General's Executive Health and Wellness Program.[6,7] Upon request of the OTSG of the Army, ICHP developed a customized model for Executive Health to address issues relevant to our nation's leaders (stress, travel and jet lag). Two interactive and educational workshops along with personalized lifestyle prescriptions for each leader and/or spouse were provided with a high level of satisfaction from the Surgeon General. Lieutenant General Patricia Horoho recognized ICHP's full support of the MHS strategic focus on Health and Wellness, when she stated that "ICHP provides a phenomenal model for initiating integrative wellness programs throughout the military. The evidence-based approach of the ICHP team compliments military medicine." In her testimony to Congress' House Appropriations Committee on April 2, 2014, LTG Horoho stated

"ICHP is the only COE that specifically addresses obstacles related to healthy living in the military. ICHP is synchronized with Army Medicine's movement to improve health."[6]

Several key personnel events impacted ICHP during this performance period. These staff additions were instrumental in our ability to move forward with our research portfolio as well as in the design of new and novel science:

- Hiring of a Cardio-Immunology Physician Consultant with an expertise in inflammatory markers as predictors of atherosclerotic disease will be instrumental in designing future ICHP protocols.
- Outcomes Data Specialist to pursue the ICHP Data Management Plan and merge data from two previous databases in order to move forward with one dataset for further analysis, including 100% quality assurance of data. This position was expanded to include responsibilities of front-desk personnel supervision and management of day to day clinic operations.
- Cardiovascular sonographer to conduct both carotid ultrasound and echocardiograms for protocols.

However, the untimely loss of 2 full time and 1 part-time Nurses Practitioners (NP) due to unexpected family illnesses and pregnancy has severely impacted ICHP's ability to see new and active patients. The average time to hire replacement NP's at WRNMMC is 9 months or longer because of security clearance which must be finished before the credentialing process starts. As a result, qualified applicants move on to other positions before clearance to start. This inefficiency hinders military research efforts greatly and is not isolated to ICHP. In ICHP, it has resulted in the establishment of a program waitlist and impacted ICHP's ability to aggressively recruit for its ongoing protocols. Hiring actions for at least 1 full time and 1 part time NP are in place. Despite the challenges, ICHP is continuing to see new participants on a limited basis.

The ICHP Executive Team was also actively engaged in finalizing the submission of the ICHP FY 2015-2019 research proposal. This submission included ongoing ICHP research but also the design of a new ICHP randomized, controlled landmark protocol with a focus on sex-differences and biomarkers as predictors of atherosclerotic disease as well as cognitive decline and cancer. With this new science, ICHP participated in dialogue with SysBioCube from USAMRMC, USACEHR, Fort Detrick, MD as a collaborative effort for data integration and metric analysis using their robust infrastructure in preparation for new protocol submission. SysBioCube is an integrated data warehouse and analysis platform for experimental data relating to diseases of military relevance. It brings together, under a single database environment, pathophysio-, psychological, molecular and biochemical data from mouse models of post-traumatic stress disorder and (pre-) clinical data from human PTSD patients. Dialogue is also progressing with LabCorp (formerly Liposcience) and Abbott Labs to participate in analysis of biomolecular work planned for this new science.

Additionally, on behalf of the Office of the Assistant Secretary of Defense (OASD), Health Affairs (HA) and the project sponsor, Dr. Terry Rauch, Director of Research & Development Policy & Oversight, OASD (HA), ICHP was invited to attend the Cardiovascular Care Capabilities-Based Assessment (CBA) Solutions Development Working Meeting as a COE. During the past year, ICHP worked with Booz Allen consultants on this project which will direct future funding (2020-2025) for Cardiovascular Care in DOD. These numerous and time

intensive meetings were held in order to help identify materiel and non-materiel shortfalls and solutions in the Joint Force's ability to research, develop, and provide cardiovascular care capabilities and innovations.

Task #1: Completion of the "*B*etter Adherence *to* *T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".

Methodology:
The purpose of this study is to determine whether knowledge of abnormal results from a noninvasive test for detection of subclinical atherosclerosis (CIMT), in addition to knowledge of CVD risk factors, enhances adherence to healthy lifestyle behaviors in comparison to only CVD risk factor knowledge. The study will be conducted with individuals at moderate to high risk for cardiovascular events based on CVD risk factor profile and evidence of significant subclinical atherosclerosis.

This two-arm, double-blinded study will randomize subjects to either receive CIMT results (R-CIMT Group) or have CIMT results withheld (W-CIMT Group) in the setting of a 3-month TLC intervention. After the 3-month TLC intervention period is completed, subjects who had CIMT results withheld will receive this information. Because knowledge of the study hypothesis could impact their behavior during the lifestyle intervention, subjects will be blinded to the study hypothesis. Similarly, research staff implementing the TLC intervention will be blinded to subjects' randomization assignment.

It is hypothesized that participants with CVD risk factors who have knowledge of their own CIMT test results showing significant subclinical atherosclerosis will demonstrate better adherence to TLC than those subjects from whom the CIMT test information is withheld. A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors.

A combined measure of adherence, reflecting both aspects of the lifestyle intervention (Mediterranean-type diet, moderate aerobic exercise), was chosen that uses accepted measures of diet and exercise adherence reported in the literature. Secondary outcomes include: 1) Adherence to each program components; 2) Changes in modifiable CVD risk factors and other biochemical markers; 3) Emotional factors such as anxiety, self-efficacy, motivation, and 4) Atherosclerosis and CIMT Knowledge Assessment Score (only in CIMT-R subjects). ***Clinical Trial Registration***—URL: www.clinicaltrials.gov. Unique identifier: NCT00458874

Findings:
Of the 1068 interested participants contacted about this study, 441 (41%) were consented and screened for participation in this study, 11% were ineligible due to low cardiovascular disease (CVD) risk profile and 47% opted out primarily for study time commitment and travel/distance reasons. Of those consenting participants, 275 (62%) screened out for the following reasons: 60% had CIMT <75 percentile for gender/age, 14% had an unacceptable past medical history, 11% withdrew consent, 6% did not meet other diagnostic or severity criteria, 1% had an intercurrent medical event during screening, and 8% were categorized as other (deployment,

relocation, job conflicts). In summary, approximately only 18% of those patients who met initial screening criteria after the telephone screen (n=948) randomized into the main study.

The remaining 166 were randomized to treatment group (83 per group); receive CIMT (R-CIMT) vs. control did not receive CIMT (W-CIMT). Thirty study cohorts yielded 142 completers vs. 24 non-completers (14.5% dropout rate); however, 161 participants had at least one clinical observation after study randomization and were included in an intent-to-treat analysis. Reasons for non-completion were: withdrew consent (4.2%), protocol non-compliance after randomization (3%), adverse events (2.4%), lost to follow-up (1.8%), and other (3%).

Study completers were predominately middle aged (mean=54.7 yrs; range 26-78), overweight (mean BMI=31.5 ± 5.6), Caucasian (48%), females (64%); however, statistical significant differences in the study groups were detected in mean age and gender. The treatment group was older (56.8 ± 9.4; p=0.015) and comprised of more women (74%; p=0.018). No differences between groups was detected in overall reported co-morbid conditions, however, over 50% of the women in the treatment group were postmenopausal. Completers were 53% hypertensive, 82% dyslipidemic, 12% Type 2 diabetes, 4% current smokers, and 56% with family history of CVD. No differences were detected between the treatment groups in their CVD risk profile.

Since assessing the impact of CIMT imaging knowledge on change in lifestyle behaviors was the primary study goal, a composite index of adherence to the lifestyle program intervention was selected as the primary outcome measure. A combined measure of adherence, reflecting both aspects of the lifestyle intervention and that uses accepted measures of diet and exercise adherence reported in the literature, was chosen. At study closeout, both groups showed marked improvement in both % of diet and exercise adherence change as compared to baseline, however, no difference was detected between the study groups ([R-CIMT] =19.6 ± 24.3 vs. [W-CIMT] = 22.6 ± 24.2); p=0.519), thereby, confirming the null hypothesis that knowledge of an abnormal CIMT scan did not have a motivational impact on overall adherence to the TLC intervention in this study.

Although the hypothesis was not supported, study completers did make significant improvements in most of their modifiable risk factors (anthropometrics; total and LDL cholesterol; triglycerides. Slight increases were seen in systolic and diastolic blood pressure. Measures of obesity including weight, BMI and % body fat were reduced by 5%. Additionally, a 5% reduction in waist circumference and a 7% reduction in abdominal sagittal diameter were seen. Both systolic and diastolic blood pressure increased by 2%. Levels of total cholesterol were reduced by 6%, LDL-cholesterol decreased by 9% and triglycerides were lowered by 14%. C-reactive protein (CRP) was decreased by 17%. Despite these positive changes, a 1% reduction in HDL-cholesterol was seen. Serum fasting glucose and insulin were collected and HOMA scores calculated as a measure of insulin resistance (IR). At baseline, 48% of the study completers had HOMA score > 2.8, indicative of IR. At study completion, 19 subjects were able to lower their HOMA scores < 2.8 and reduce their risk of pre-diabetes. Overall, serum glucose was reduced by 4% and fasting insulin was reduced by 23.3%.

Although these data do not support the motivational impact of CIMT imaging on program adherence, it is clear that this data supports participation in a multi-faceted lifestyle change

which includes intensive education, frequent monitoring and group support. Participation resulted in substantial CVD risk factor improvements. Some of these changes rival what has been observed with pharmacological treatment.
In addition to the main study, a formative evaluation of the lifestyle program intervention took place almost 2 years after study completion for many study participants. Of the 140 surveys mailed to consenting BATTLE Study participants, 49% (n=68) were returned. Additionally, 35 telephonic interviews (31 year 2 completers; 4 non-completers) were conducted on consenting participants over a 2 month period. Survey item responses have been collated and all telephone interviews have been transcribed. Identification of common theme from telephone interviews and open-ended survey response continues.

Protocol Deviation/Adverse Events:
One protocol deviation was reported to the WRAMC Department of Clinical Investigations (DCI) Human Use Committee (HUC) during this study and previously reported. During the course of this study, 10 serious (SAEs) and 25 non-serious AEs have been reported to WRAMC HUC.

Status:
This task has been completed. Data analysis completed and final study documentation received back from PREMIER CRO in December 2011. Study closure documents approved by WRNMMC Department of Research Programs (DRP) Institutional Review Board (IRB) on 25 October 2013 and forwarded to USAMRMC Office of Research Protections (ORP) Human Research Protection Office (HRPO). Manuscripts are being finalized for submission.

Abstracts Presented

Modlin RE, Walizer EM, Vernalis MN. CIMT imaging knowledge effect on lifestyle program adherence. TriService American College of Physicians (ACP), Bethesda, MD, November 2012. (Podium)

Abstract

Introduction: The use of carotid intima media thickness (CIMT) ultrasound to identify subclinical atherosclerosis is widespread, but few studies examine its influence on patient behavior. We evaluated the use of CIMT imaging knowledge to motivate adherence to a lifestyle program.
Hypothesis: We hypothesized participants with cardiovascular disease (CVD) risk factors who have knowledge of their CIMT test results will demonstrate better program adherence than those participants from whom the CIMT test information was withheld.
Methods: Participants, with ≥ 2 CVD risk factors and CIMT measurements ≥ 75th percentile for age, were randomized into either the intervention group [receive results (R-CIMT)] or control group [withhold results (W-CIMT)]. The R-CIMT group received their CIMT image weekly. All participants received the 12-week program (Mediterranean diet, aerobic exercise, group support). We determined the overall change in program adherence from baseline to week 12 or last observation carried forward using an ANCOVA model with CIMT group and gender as factors and age as the covariate. Percent adherence was calculated as a composite measure of diet and exercise adherence at baseline and 12 weeks [Diet adherence =

(Mediterranean Diet Score/14) X 100% and Exercise adherence = (weekly exercise time/180) X 100%]. Adherence measures were capped at 100%. R-CIMT group received a CIMT tutorial explaining results and associated CVD risk. Comprehension was assessed by a knowledge test.
Results: 161 participants (mean age=53.6 ± 10.8; 62% women; 48% black) were enrolled over 2 years. No differences were seen between groups in baseline demographics, except W-CIMT group was younger (52 vs. 55 yrs; p=0.049). When comparing R-CIMT vs. W-CIMT groups, no difference was detected in overall % change in adherence (16.4 ± 25.6 vs. 19.8 ± 25.4; p=0.392). The median knowledge test score was 90% (80,100) in the participants responding (66%).
Conclusions: In conclusion, although the presence of subclinical atherosclerosis increased participant knowledge of their increased CVD risk, it did not motivate participants to make more lifestyle changes than those in the control group.

Walizer EM, Vernalis MN, Modlin RE. Adherence to a lifestyle intervention program not improved by visual knowledge of carotid intima atherosclerosis. *Circ Cardiovasc Qual Outcomes* 2013;6:A43.

American Health Association's (AHA) Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2013 Scientific Session, Baltimore, MD, May 2013. (Poster)

Abstract

Background: The use of carotid intima media thickness (CIMT) ultrasound to identify subclinical atherosclerosis is widespread, but few studies examine its influence on patient behavior. This randomized, double-blinded clinical trial examined, in participants with ≥ 2 cardiovascular disease (CVD) risk factors and subclinical atherosclerosis, the use of CIMT ultrasound images to motivate adherence to a lifestyle change program.
Methods: Participants were randomized into either the intervention group [receive CIMT results weekly (R-CIMT)] or control group [CIMT results withheld (W-CIMT)]. All participants received the 12-week lifestyle program (Mediterranean diet, aerobic exercise, group support). We determined the overall change in program adherence from baseline to week 12 using an ANCOVA model with CIMT group and gender as factors and age as the covariate. Percent adherence was calculated as a composite measure of diet and exercise adherence at baseline and 12 weeks [Diet adherence = (Mediterranean Diet Score/14) X 100% + Exercise adherence = (weekly exercise time/180) X 100%]/2 where exercise was capped at 100%. The R-CIMT group received a CIMT tutorial explaining results and associated CVD risk. Comprehension was assessed by a knowledge test at week 12 in the R-CIMT group only. Baseline motivation was assessed to determine its predictive ability of adherence when added to group assignment in a standard regression model.
Results: 142 participants (R-CIMT n=69; W-CIMT n=73) completed the study; mean age = 55 ± 10 yrs; 64% women (n=91); 45% black (n=64). Several demographic differences were seen between groups: R-CIMT group was older (57 vs. 53; p=0.02) and had a higher % female [74 (51 of 69) vs. 55 (40 of 73); p=0.02]. When comparing R-CIMT vs. W-CIMT group assignment, no difference was detected in overall % change in adherence respectively (19.6 ± 24.3 vs. 22.6 ± 24.2; p=0.52). Baseline motivation was similar in both groups (R-CIMT=4.6 ± 0.6; W-CIMT=4.5 ± 0.6) and not predictive of change in adherence when added to group assignment (p=0.68). Median knowledge test score = 90% (80,100) in responding completers (80%; 55 of

69).
Conclusions: Although the presence of subclinical atherosclerosis increased participant knowledge of their increased CVD risk, it did not act as a motivator in these participants to improve lifestyle change adherence more than those in the control group.

Walizer EM, Vernalis MN, Modlin RE. Influence of CIMT as a motivator for health behavior change in a heart health program. *Circulation.* 2014;129(Suppl_1):AP126.

AHA Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2014 Scientific Session, San Francisco, CA, March 2014. (Poster)

Abstract

Introduction: Carotid intima media thickness (CIMT) ultrasound is a known surrogate marker of atherosclerosis but few studies examine its influence on patient behavior. Motivation and self-efficacy (SE) are known predictors of health behavior change. This randomized, double-blind trial examined 1) use of CIMT images plus associated CVD risk to motivate adherence, and 2) the predictive ability of motivation and SE on adherence change.

Methods: Patients with ≥ 2 cardiovascular disease (CVD) risk factors and subclinical atherosclerosis were assigned to either the intervention group [receive results weekly (R-CIMT)] or control group [withhold results (W-CIMT)]. All patients received a 12-week lifestyle program (Mediterranean diet, aerobic exercise, group support). Overall change in adherence from baseline to week 12 was determined using an ANCOVA model where % adherence was a composite measure of diet and exercise adherence. Initial motivation plus exercise and nutrition SE were assessed to determine their predictive ability of adherence in a standard regression model.
Results: 166 patients randomized; 161 (R-CIMT n=81; W-CIMT n=80) eligible for intention-to-treat analysis. Patients were middle age (mean age = 54 ± 11 yrs), 62% (100 or 161) women, 48% (77 of 161) black. Baseline group differences: W-CIMT group was younger (52 vs 55 yrs; p=0.05), had a lower systolic blood pressure (120 vs 125; p=0.01), lower % family history of CVD (49 vs 65; p=0.03). In comparing R-CIMT vs W-CIMT groups, no difference was detected in overall % adherence change (16.4 ± 25.6 vs 19.8 ± 25.4; p=0.39). Initial motivation and SE measures were not predictive of change in adherence when added to group assignment (see Table).

	P-Value (Regression Coefficient)			
Model	CIMT Group	Exercise SE	Nutrition SE	Initial Motivation
Adherence Change* = CIMT group + Initial Motivation	0.443			0.677
Adherence Change* = CIMT group + Exercise SE[1] + Nutrition SE[1]	0.414	0.927	0.784	
Adherence Change* = CIMT group + Initial Motivation + Exercise SE[1] + Nutrition SE[1]	0.469	0.798	0.575	0.552

*Adherence change was computed by adherence (capped at 100%) at week 12.
[1]Baseline exercise and nutrition SE are considered covariates.

Conclusions: CIMT evidence of subclinical atherosclerosis increased participant CVD risk awareness but did not translate into actionable healthy behavior changes beyond those in the control group. Neither exercise nor dietary adherence was affected by initial motivation or self-efficacy when added to CIMT risk awareness.

Task #2: Completion of the "CADRe Five-Year Follow-up" Protocol.

Methodology:
This follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control results after their original CADRe study participation. This study will continue as a longitudinal observational study where patients will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. This study will involve prospective collection of data. All collected data is considered WRNMMC Cardiology standard of care for the study population identified.

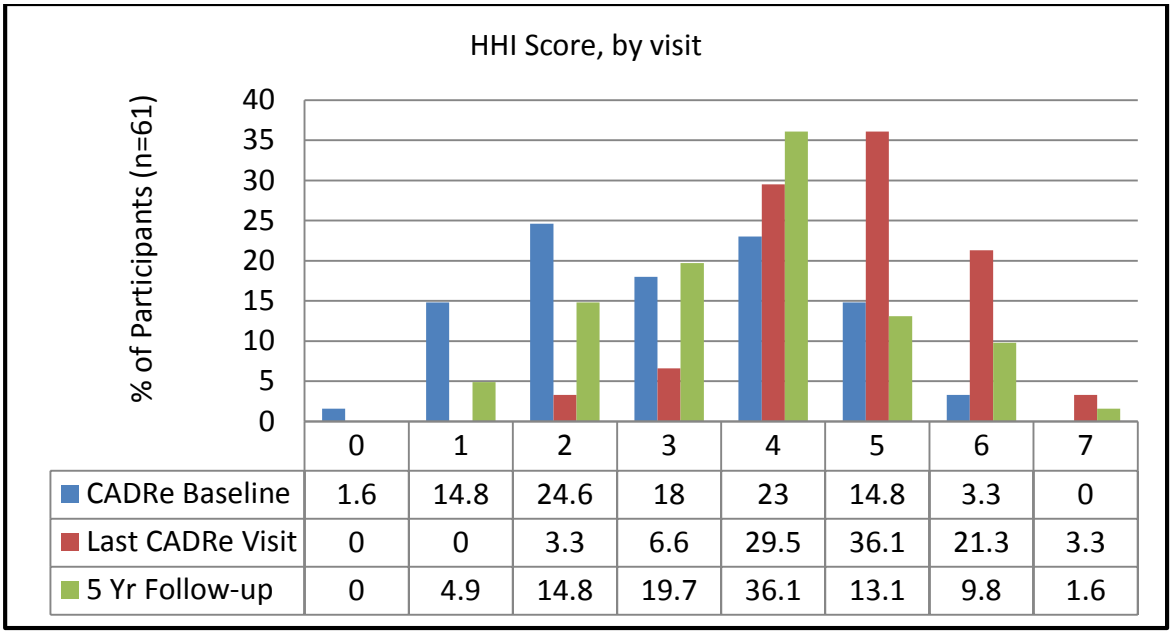
It is hypothesized that participants who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control. Up to 163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) were re-contacted and invited to participate in this 5-year follow-up study (post-study completion or expected completion).

A composite index of 7 heart healthy characteristics (BMI 18.5 – 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise ≥ 150 min/week, and daily practice of CADRe program stress management techniques) was selected as the primary outcome measure since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The Heart Health Index (HHI), presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable. Secondary outcome measures include: Changes in modifiable CVD risk factors (blood pressure, body composition and fitness, lipid levels and glucose); C-reactive protein and, Quality of Life.

Findings:
Of the 163 eligible CADRe study patients, 76 provided informed consent and made at least 1 follow-up visit. Of those 76 participants, 64 provided study year 5 data with 61 of these participants CADRe study completers. In this report, we will present the data from the 61 CADRe study completers. Upon entry into the original CADRe study, participants were predominately middle-aged (mean age: 61 ± 10; range 36 to 80 years old), male (69%), Caucasian (77%) and retired military (63%). Most participants have known coronary artery disease (61%), hypertension (53%) and hypercholesteremia (80%). Only 14% of the participants were diabetic.

Figure 1 shows the distribution of index measures achieved by CADRe study completers at each comparison time point (CADRe study baseline, last CADRe study visit and Year 5 follow-up visit). At CADRe baseline, the largest percent of participants (25%) met 2 of the

HHI components. By CADRe study completion, 61% were able to meet at least 5 index measures. Five years later, 61% of study completers were able to maintain at least 4 heart healthy behaviors.



	0	1	2	3	4	5	6	7
CADRe Baseline	1.6	14.8	24.6	18	23	14.8	3.3	0
Last CADRe Visit	0	0	3.3	6.6	29.5	36.1	21.3	3.3
5 Yr Follow-up	0	4.9	14.8	19.7	36.1	13.1	9.8	1.6

Figure 1. Distribution of participants by number of index measures achieved, by visit (%).

The distribution of participants achieving the individual index measures at each study time point is reflected in Figure 2. During the CADRe study, daily stress management practice was the program component (1 hour daily) that was most difficult for participants to adhere during the 1 year program. At the 5-year follow-up, more participants reported incorporation of daily stress management techniques than at the last CADRe study visit, but at a 27% reduction in minutes/week (see Table 1). The percent of participants achieving at least 150 minutes of exercise/week at the 5-year visit was significantly lower than upon CADRe study completion but slightly higher than reported at baseline. The proportion of participants achieving the blood pressure measure was similar across all time points. No significant change was seen in the proportion of participants meeting the consumption of ≥ 5 fruits and vegetables per day and LDL cholesterol < 100 mg/dL between the 5-year follow-up visit and study completion, however, achievement of these measures was significantly better than at baseline. Almost 50% fewer participants were able to achieve dietary fiber intake ≥ 25 grams per day at the 5-year follow-up visit than study completion. The proportion of participants achieving the dietary intake measure was similar at baseline and 5-year follow-up. Finally, about one-half of CADRe participants were able to obtain a normal BMI at study completion as compared to 28% at baseline. At the 5-year follow-up visit, fewer participants were able to meet the BMI measure than at baseline.

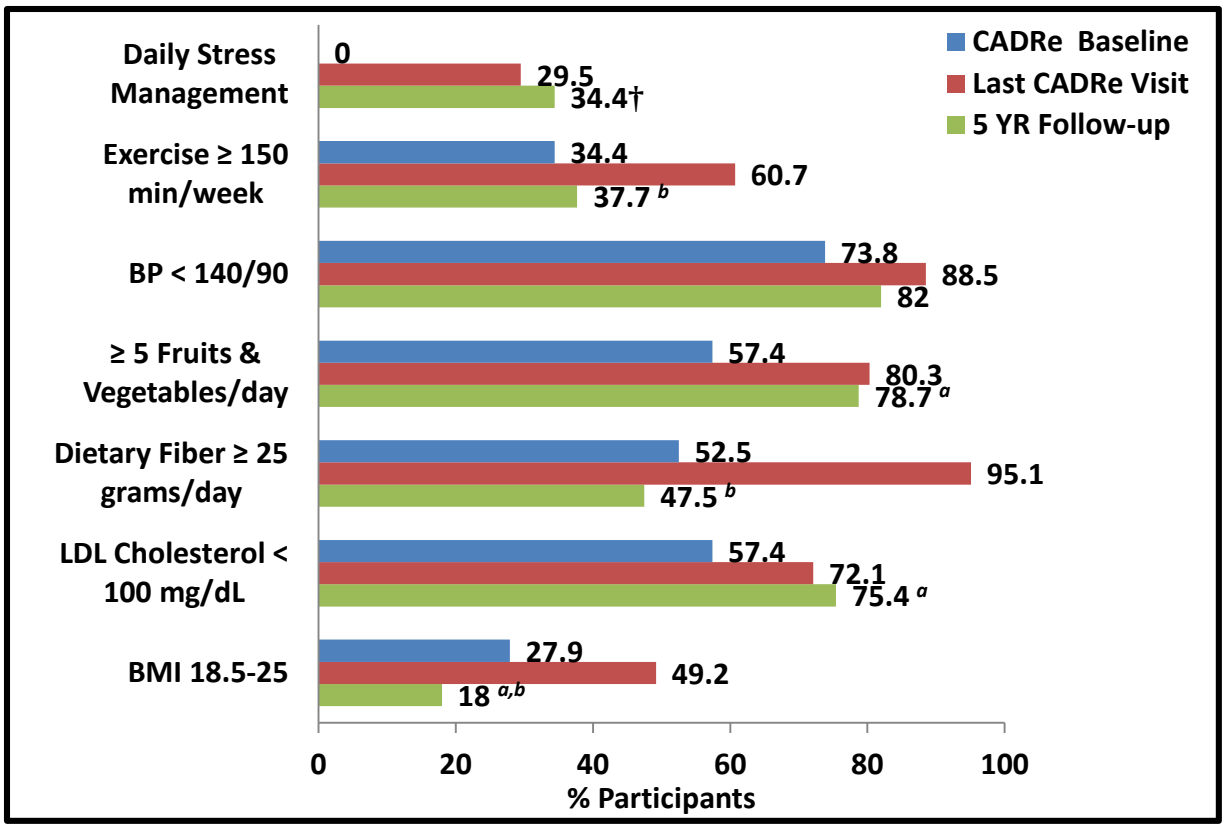


Figure 2. Distribution of participants achieving individual index measures, by visit (%).
[a]p < 0.05; comparing 5-year follow-up visit to CADRe baseline from McNemar's test
[b]p < 0.05; comparing 5-year follow-up visit to last CADRe visit from McNemar's test
†A p value could not be computed as there were no discordant pairs (last CADRe visit vs. baseline)

Table 1 summarizes the individual HHI measures 5 years post CADRe study completion as compared to CADRe baseline and last CADRe study visit. Although there is a significant difference in the mean 5 Year HHI score when compared to mean HHI score at last CADRe study visit, findings do suggest persistence of several heart healthy behaviors. Participants reported persistence of several behaviors: 1) fruit and vegetable intake ≥ 5 servings per day; 2) weekly exercise ≥ 150 minutes per week, and 3) LDL cholesterol < 100 mg/dL. Conversely, participants were not able to sustain gains made while in the CADRe study in regards to daily fiber intake, weekly stress management practice, BMI and systolic/diastolic blood pressure. However, participants did not return to reported baseline measurements, except for a 3% increase in BMI, despite a reported 10% increase in exercise minutes since leaving the CADRe study. Since exercise minutes were self-reported during the CADRe study and follow-up visit, participants may have not accurately reflected their time or level of activity (aerobic vs. nonaerobic). The additional 5% reduction in LDL cholesterol from last CADRe visit to the 5-Year follow-up visit is not statistically significant and may be attributed to the majority of participants (90%) on lipid-lowering medications with an increase or no change in these medications (89%). Similarly, antihypertensive medications were being taken by 91% of the participants. No change or an increase in hypertension medications over the 5 year follow-up period was reported by 87% of participants. This coupled with a 5 year age

increase could account for significant increase (4%) in systolic blood pressure. Diastolic blood pressure was relatively stable with a 3% increase.

Table 1. Summary of individual HHI measures, 5-Yr follow-up visit vs CADRe baseline, last CADRe visit.

	CADRe Baseline	Last CADRe Visit	5-Yr FU	Change from Baseline	P	Change from Last CADRe Visit	P
HHI Score	3.0 ± 1.5	4.8 ± 1.1	3.7 ± 1.4	0.7 ± 1.5	0.0005	-1.0 ± 1.5	<0.0001
Dietary Fiber (grams)	28.8 ± 14.2	47.9 ± 16.5	26.1 ± 13.4	-3.2 ± 11.6	0.043	-22.2 ± 15.8	<0.0001
Fruit & Vegetable (servings)	5.8 ± 3.4	7.3 ± 3.5	8.8 ± 5.0	3.0 ± 5.1	0.0001	1.5 ± 5.4	0.029
Exercise Time (minutes/week)	134.3 ± 136.9	168.2 ± 70.8	184.3 ± 252.0	50.0 ± 248.8	0.117	16.1 ± 262.9	0.634
Stress Management Time (minutes/week)	21.7 ± 53.1	227.0 ± 128.2	165.2 ± 177.1	143.5 ± 179.5	<0.0001	-61.8 ± 182.1	0.010
BMI	29.0 ± 6.0	27.4 ± 6.1	29.9 ± 6.7	0.9 ± 3.3	0.030	2.5 ± 3.5	<0.0001
LDL Cholesterol (mg/dL)	97.8 ± 31.9	86.1 ± 23.6	81.2 ± 23.3	-16.6 ± 27.7	<0.0001	-4.9 ± 20.4	0.064
Systolic BP (mmHg)	128.0 ± 15.1	120.3 ± 12.7	125.3 ± 14.8	-2.7 ± 18.4	0.255	5.0 ± 14.4	0.008
Diastolic BP (mmHg)	71.5 ± 9.3	69.3 ± 7.5	71.1 ± 7.6	-0.4 ± 9.2	0.739	1.8 ± 9.1	0.128

Values are mean ± SD.

A comparison of secondary biochemical measures across the 3 study time points can be found in Table 2. Serum lipids, fasting glucose and C-reactive protein measures remained fairly stable over time if not improved at the 5-year follow-up when compared to the 5-year follow-up visit. These findings are most likely related to ease of access to care and use of lipid-lowering medications in the majority of participants. However, participant did report a significant increase (8%) in mean weight (kg) and % body fat (20%) at 5-year follow-up when compared to study completion. Baseline and 5-year follow-up weights were comparable, but the 11% difference in % body fat between baseline at 5-year was significant.

Table 2. Summary of secondary variables, 5-Yr follow-up visit vs CADRe baseline, last CADRe visit.

	CADRe Baseline	Last CADRe Visit	5-Yr FU	Change from Baseline	P	Change from Last CADRe Visit	P
Weight (kg)	86.8 ± 22.0	82.2 ± 22.8	88.8 ± 25.4	2.0 ± 8.6	0.072	6.6 ± 9.8	<0.0001
Body Fat (%)	27.5 ± 8.8	25.3 ± 8.9	30.5 ± 9.1	2.5 ± 4.9	0.0003	4.8 ± 4.3	<0.001
Fasting Glucose (mg/dL)	102.1 ± 18.4	98.0 ± 19.1	96.6 ± 18.3	-5.5 ± 18.4	0.022	-1.5 ± 19.7	0.566
Total Cholesterol (mg/dL)	168.6 ± 41.2	157.5 ± 32.1	150.0 ± 30.0	-18.7 ± 35.7	0.0001	-7.5 ± 28.8	0.046
HDL Cholesterol (mg/dL)	48.5 ± 12.1	45.8 ± 10.2	48.7 ± 12.9	0.2 ± 11.3	0.901	2.9 ± 11.0	0.046
Triglycerides (mg/dL)	145.9 ± 84.2	159.8 ± 88.4	131.3 ± 85.0	-14.6 ± 79.7	0.157	-28.6 ± 74.4	0.004
C-reactive protein (mg/dL)	0.32 ± 0.44	0.22 ± 0.27	0.23 ± 0.41	-0.10 ± 0.46	0.110	-0.001 ± 0.37	0.988

Values are mean ± SD.

After exposure to a yearlong intensive lifestyle program incorporating a vegan diet, moderate aerobic exercise, stress management, group support and case management, participants were able to achieve a higher proportion of heart healthy behaviors at study completion. After 5 years, the proportion of participants achieving most behaviors was not sustained, but did not return to baseline levels. Healthy behaviors where access to medical care and use of medications may have played a role (blood pressure and LDL cholesterol) were sustained. Persistence of those heart health behaviors that required a long-term commitment to lifestyle change (exercise, stress management practice, intake of dietary fiber, weight control) were more difficult to sustain in this long-term study.

Adverse Events:
There were 2 adverse events (AEs) reported and a summary was provided to the WRAMC HUC.

Status:
This task is completed. Final data analysis along with additional ad hoc data analysis was received from CLINIRX under a statistical contract. Study closure documents were approved by WRNMMC DRP on 12 Mar 13 and acknowledged by USAMRMC ORP HRPO on 17 May 13.

Task #3: Continuation of the "Comprehensive Cardiovascular Risk Assessment and Health Program (CHP)" at WRNMMC.

Methodology:
This program serves as a platform for ongoing translational research activities, a "virtual laboratory" based on scientific findings for the development of best personalized preventive practices. In other words, the platform allows ICHP to gather an expansive number of data points for each patient or subgroup of patients (eventually combined with data at a molecular level) that when leveraged will result in the creation of new tools in technology to define wellness, predict and prevent disease, and empower patients and providers to transform their healthcare.

The CHP platform has a dual purpose and is multifunctional. This platform 1) allows for multiple research protocols to be conducted as it sets the stage for recruitment, enrollment and hypothesis generation, advanced data modeling and simultaneously, and; 2) provides a venue where research findings from these protocols can then be tested, validated and translated into application for clinical practice. Our protocols within the CHP are specifically designed to examine the effects of our military's high op tempo which predisposes our service members to accelerated atherosclerotic risk resulting from high stress, PTSD, depression, sleep insufficiency, overweight, prediabetes and prehypertension among other traditional disease risk factors.

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) along with tailored and personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists, stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up

to 1000 patients may be enrolled each year. Some of the patients (such as nurses or traumatic injury patients, etc.) may be in subgroup programs because of unique needs. The CHP serves as a platform for ongoing translational research activities, a "virtual laboratory" for the development of best preventive practices and for CV educational and marketing materials.

Status:
This task is ongoing.

The "Outcomes of the Cardiovascular Prevention Program (CPP)" protocol provided for retrospective examination of existing data for the purpose of examination and reporting of the results of the evaluations and interventions of the CHP. With the initiation of the CHP Registry protocol (see Sub Task 3.3), it was determined that this protocol no longer should remain active. Therefore, a closure report was submitted to WRNMMC DRP on 29 November 2015 and approved 29 January 2016. The closure documents and acknowledgement letter were forwarded to USAMRMC HRPO on 4 February 2016. Data from this "retrospective" database protocol will now be included as part of the CHP Registry protocol.

The following manuscripts/abstracts during this period of performance are attributed to the above protocol.

Manuscripts Published:

Kashani M, Eliasson A, Vernalis M. Perceived stress correlates with disturbed sleep—a link connecting stress and cardiovascular disease. *Stress* 2012 Jan;15(1):45-51. doi: 10.3109/10253890.2011.578266. Epub 2011 Jun 19. ***Cited by 8 PubMed Central articles.***

Manuscript Abstract

The association between stress and risk of cardiovascular disease (CVD) is becoming established. A mechanistic link clarifying the intermediate steps between the experience of stress and the development of CVD would support this association. We sought to examine the role of perceived stress as a factor associated with disturbed sleep with the goal of providing an explanation for the stress-CVD connection. We performed a cross-sectional analysis of data recorded by subjects at entry to our CVD prevention program. Data collection included questionnaire surveys, anthropometrics, and a CVD-relevant laboratory panel. Of 350 consecutively enrolled subjects (mean age 54.4 $\pm$ 12.4 [SD] years, 138 men, 39%); 165 (47%) scored above the mean for stress measures. These high-stress subjects displayed an increased cardiovascular risk profile including elevated body mass index (mean $\pm$ SD 31.1 $\pm$ 5.9 vs. 29.0 $\pm$ 5.9, $r_s = 0.175$), increased waist circumference (102 $\pm$ 17 cm vs. 98 $\pm$ 14, $r_s = 0.135$), and elevated high sensitivity serum C-reactive protein (0.384 mg/dl vs. 0.356, $r_s = 0.109$). High-stress subjects also demonstrated greater daytime sleepiness (Epworth Sleepiness Scale: 10.4 $\pm$ 5.0 vs. 7.8 $\pm$ 4.8, $r_s < 0.316$), greater fatigue (fatigue scale: 5.4 $\pm$ 2.2 vs. 3.4 $\pm$ 2.4, $r_s = 0.484$), poorer sleep quality (Pittsburgh Sleep Quality Index: 8.5 $\pm$ 4.4 vs. 5.9 $\pm$ 4.0, $r_s = 0.416$), and shorter sleep duration (20 min less/24 h, r_s = negative 0.177) with a higher risk for sleep apnea (60% at high risk vs. 40%, $p = 0.003$) than low-stress subjects. High stress is associated with significant disturbances in sleep duration and sleep quality. Stress levels also correlate with daytime

consequences of disturbed sleep. The stress-sleep connection may be an important mechanistic mediator of the association between stress and CVD.

Eliasson AH, Kashani MD, Howard RS, Vernalis MN, Modlin RE. Fatigued on Venus, sleepy on Mars - gender and racial differences in symptoms of sleep apnea. *Sleep Breath*. 2015 Mar;19(1):99-107. doi: 10.1007/s11325-014-0968-y. Epub 2014 Mar 15. ***Cited by 1 PubMed Central article.***

Manuscript Abstract

Objective: Clinical guidelines for the care of obstructive sleep apnea (OSA) recommend evaluation of daytime sleepiness but do not specify evaluation of fatigue. We studied how subjects with and without OSA experience fatigue and sleepiness, examining the role of gender and race.

Design, setting, patients: Consecutive subjects entering our heart health registry completed validated questionnaires including Berlin Questionnaire for OSA, Fatigue Scale, and Epworth Sleepiness Scale. Data analysis was performed only with Whites and Blacks as there were too few subjects of other races for comparison.

Results: Of 384 consecutive subjects, including 218 women (57 %), there were 230 Whites (60 %) and 154 Blacks (40 %), with average age of 55.9 ± 12.8 years. Berlin Questionnaires identified 221 subjects (58 %) as having high likelihood for OSA. Fatigue was much more common in women (75 %) than in men (46 %) with OSA ($p < 0.001$), while frequency of fatigue was similar in women (30 %) and men (29 %) without OSA ($p = 0.86$). In multivariate analysis, men with OSA were sleepier than women; Black men with OSA had higher Epworth scores (mean ± SD, 12.8 ± 5.2) compared to White men (10.6 ± 5.3), White women (10.0 ± 4.5), and Black women (10.5 ± 5.2), $p = 0.05$. These gender differences were not related to the effects of age, body mass index, perceived stress, sleep duration, or thyroid function.

Conclusions: Women report fatigue more commonly with OSA than men. Men experience sleepiness more commonly with OSA than women. The findings suggest that evaluation of sleep disorders must include an assessment of fatigue in addition to sleepiness to capture the experience of women.

Abstracts Presented:

Eliasson A, Kashani M, Vernalis M. Fatigued on Venus, sleepy on Mars? *Am J Respir Crit Care Med* 2012;185:A5033.

American Thoracic Society (ATS) Scientific Meeting, May 2012, San Francisco CA. (Poster)

Abstract**

Rationale: Subjective sleepiness and fatigue are recognized as separate symptoms which may occur singly, together, or may both be absent in subjects with obstructive sleep apnea (OSA). The inter-individual experiences of sleepiness and/or fatigue have recently been shown to be stable and trait-like with potential genetic causes. We sought to examine the vulnerabilities for sleepiness and fatigue in subjects with and without sleep apnea with special attention to the role of gender and race.

Methods: Consecutive subjects entering our heart health program completed a series of validated questionnaires. Thyroid function was tested in every subject. Sleepiness was defined by Epworth Sleepiness Scale $\geq$ 10 of 24 points. Fatigue was defined by the Stanford Fatigue Visual Analog Scale $\geq$ 5 of 10 points. The Berlin Questionnaire identified subjects as high or low likelihood for OSA. The two groups were compared using Fisher's exact test and two sample t-test as appropriate. For data analysis by race, comparisons were limited to White and Black categories as there were too few subjects for other comparisons.
Results: Of 295 consecutive subjects, 172 women (58%), there were 172 Whites, 105 Blacks, 13 Hispanics, 2 Asians and 3 others, with average age 57.4$\pm$12.7 years. Sleepiness was found in 129 subjects (44%) and fatigue in 90 subjects (31%). Berlin Questionnaires identified 159 subjects (54%) as high likelihood for OSA. There was no difference in thyroid function between subjects with and without a positive Berlin score ($p=0.52$). Without OSA present, numbers of subjects with fatigue were similar in women (15%) and men (20%), $p=0.63$. With OSA, fatigue was much more common in women (57%) compared to men (26%), $p<0.001$. For sleepiness, there was no significant difference between the genders, $p=0.43$, but Black men did demonstrate a significant increase in subjects with sleepiness when comparing those with no OSA (29%) to those with OSA (86%), $p=0.05$.
Conclusions: Symptoms of fatigue and sleepiness are reported with different prevalence according to gender and race. Overall, women report fatigue more commonly in association with OSA than men. Black men experience sleepiness more commonly with OSA than other groups. These differences are not related to thryoid function. These findings deserve explanation with research that incorporates an objective measure of sleepiness and includes a broader range of variables such as the effects of total sleep time, co-morbid conditions, and medications.

**This abstract was also presented at the TriService American College of Physicians (ACP), Bethesda, MD, November 2012. (Podium)

Kashani M, Eliasson A, Bailey K, Vernalis M. Novel stress reduction technique improves sleep and fatigue. *Chest* 2012;142(4_MeetingAbstracts):1052A.

American College of Chest Physicians Scientific Meeting, Atlanta, GA, October 2012. (Podium)

Abstract

Purpose: A growing body of evidence substantiates the important roles of stress and sleep in cardiovascular disease. We sought to determine the effect of a brief, portable stress reduction technique, the ten-minute Tension Tamer on improvement of stress levels and sleep parameters in a heart health program.
Methods: Adult men and women self-referred or referred to the Integrative Cardiac Health Project were assessed for levels of perceived stress and sleep quality using validated surveys. Subjective stress was measured using the Perceived Stress Scale (PSS14, total possible points 56); sleep quality was evaluated with the Pittsburgh Sleep Quality Index (PSQI, total possible points 21); fatigue was assessed using the 10 point fatigue scale. After a 30-minute introductory workshop, subjects were given instruction and guided opportunities to practice ten-minute Tension Tamers over the course of four 30-minute visits with a stress management specialist. This brief technique, encouraged at bedtime, involves deep breathing and imagery using the

subject's personal preference. Upon completion of the four visit practice sequence, validated surveys were reassessed and compared with baseline values using t-tests.
Results: Of 334 subjects (mean age 55.7 years, 135 men, 200 Caucasian, 117 African-American, 14 Latino, 3 other), 218 (65%, mean age 56.6 years, 40% men) improved their perceived stress by 6.6 points ($p<0.001$) using the Tension Tamer technique. Non-improvers, 116 subjects (34%, mean age 59.7, 41% men) showed worsened stress levels by 4.6 points. Comparing Improvers with Non-Improvers showed significant differences in sleep quality (PSQI improved 1.78 vs worsened 0.89 points, $p<0.001$), decreased sleep latency (decreased 4 vs increased 1.9 minutes, $p=0.04$), and decreased fatigue (decreased 0.89 vs increased 0.27 points, $p<0.001$).
Conclusion: A novel stress reduction technique, the ten-minute Tension Tamer, can reduce perceived stress levels in a majority of subjects resulting in improved sleep quality, decreased sleep latency and improved fatigue.
Clinical Implications: Using a portable stress reduction technique in short intervals may be a unique approach to improve cardiovascular risk through sleep improvement.

This abstract received the following Press Coverage from numerous media sites:
https://consumer.healthday.com/mental-health-information-25/behavior-health-news-56/10-minute-tension-tamer-at-bedtime-may-help-you-sleep-study-669909.html

http://health.usnews.com/health-news/news/articles/2012/10/23/10-minute-tension-tamer-at-bedtime-may-help-you-sleep-study

http://www.news-medical.net/news/20121022/Simple-10-minute-Tension-Tamer-could-help-relieve-stress-and-decrease-fatigue.aspx

http://indianexpress.com/article/lifestyle/10minute-tension-tamer-can-reduce-stress-and-improve-sleep/

http://www.altmedicinezone.com/alternative-treatment/a-technique-to-manage-insomnia/

http://isp.netscape.com/whatsnew/package.jsp?name=fte/10minutesleep/10minutesleep

http://www.usmedicine.com/agencies/department-of-defense-dod/quick-stress-reduction-technique-improves-sleep-for-heart-patients/

http://www.huffingtonpost.com/2012/10/26/stress-sleep-insomnia_n_2019418.html

http://www.business-standard.com/article/pti-stories/10-minute-tension-tamer-can-reduce-stress-and-improve-sleep-112102200358_1.html

http://www.daily-frontier.com/Frontier/2012075521-10-minute+tension+tamer+can+reduce+stress+and+improve+sleep.html

http://www.thehealthsite.com/news/reduce-your-stress-levels-with-ten-minute-tension-tamers/

https://www.highbeam.com/doc/1G1-309160284.html

Eliasson AH, Kashani M, Bailey K, Vernalis M. Sleep quality improves in adherents to heart health program without change in sleep duration. *SLEEP* 2013;36:A296-297.

Affiliated Professional Sleep Society Meeting (APSS), Baltimore, MD, June 2013. (Poster)

Abstract

Introduction: The Integrative Cardiac Health Project (ICHP) is a 6-month heart health program that promotes comprehensive lifestyle change including exercise, the Mediterranean Diet, and use of practical stress management techniques. Subjects are also educated on the principles of good sleep hygiene. Among subjects adhering to suggested lifestyle changes, we queried what the impact would be on their sleep duration, sleep quality and symptoms of fatigue. Since serum triglycerides respond rapidly to lifestyle changes, this lab test may be used as a marker of adherence to the program. We therefore analyzed sleep parameters in the cohort of adherents (triglyceride improvers) among ICHP program graduates.
Methods: At program entry, participants complete validated questionnaires, specifically the Pittsburgh Sleep Quality Index (PSQI), fatigue visual analog scale and Perceived Stress Scale (PSS). Subjects also submit to blood measurement of a cardiac-relevant lab panel. Differences between values at program entry and completion were compared using paired t-test. Analysis was limited to subjects whose triglycerides improved.
Results: Of 410 consecutive finishers (mean age 58 yrs, 40% men), 240 (59%) showed improvements in triglycerides (146 to 100, $p < 0.001$). Other lipids improved (TC 189 to 179, $p=0.003$; LDL 111 to 103, $p=0.007$; HDL 55 to 59, $p=0.01$). Glucose (105 to 91, $p<0.001$), insulin (14 to 10, $p=0.04$) and HOMA (3.7 to 2.3, $p=0.007$) improved. Blood pressure also improved (SBP 128 to 125, $p=0.02$; DBP 80 to 78, $p=0.002$). Lengthening of sleep duration was not statistically significant (6.3 to 6.5 hours/night, $p=0.075$) and fatigue did not change significantly 4.2 to 3.8, $p=0.085$). However, PSQI did show significant improvement (7.31 to 6.36, $p=0.01$) and PSS decreased (22 to 19, $p<0.001$).
Conclusions: Successful adherence to a comprehensive lifestyle change program without a specific focus on increasing total sleep time may improve sleep quality even without significant changes in sleep duration.

Eliasson AH, Kashani MD, Doody MM, Jones MK, Vernalis MN. Fatigue in women is a key symptom in evaluation of sleep apnea. *Chest.* 2014;146(4_MeetingAbstracts):938A.

CHEST 2014 Scientific Session, Austin, TX, October 2014. (Poster)

Abstract

Purpose: Recently published guidelines for management of obstructive sleep apnea (OSA) endorse evaluation of sleepiness with the Epworth Scale but do not suggest the assessment of fatigue. Prior research on gender differences in OSA symptoms has shown conflicting results in part because symptom questionnaires have not included fatigue and in part because OSA was determined by screening questionnaire not by gold standard overnight polysomnography. We sought to clarify if symptoms differed by gender in subjects with OSA confirmed by overnight polysomnography utilizing symptom-specific questionnaires.

Methods: Of subjects entering a cardiovascular disease prevention registry, we gathered data on demographics and sleep-related symptoms for consecutive patients who underwent diagnostic polysomnography. OSA was defined with a respiratory disturbance index (RDI) of ≥ 5 events per hour. Sleepiness was recorded using the Epworth Scale (ES, range 0 to 24). Fatigue was measured with the Stanford Fatigue Scale (FS, range 0 to 10). Subjects with and without OSA were compared by gender for symptoms of fatigue and daytime sleepiness by t-test.
Results: Of 62 consecutive subjects (40 women, mean age 57.4±12.6 years) ES was 10.0±4.7, FS was 4.9±1.9. With no OSA, ES in women (8.2±4.8) was not different from men (9.5±4.0, p=0.63) and FS in women (5.4±2.0) was not different from men (5.0±3.2, p=0.79). However, with OSA, ES in women (10.4±5.3) was similar to men (10.5±5.0, p=0.93) but FS in women (5.4±1.8) was significantly higher than for men (4.1±1.8, p=0.03). This greater degree of fatigue in women with OSA was found despite a lack of statistical difference in polysomnographical variables between women and men for RDI (p=0.42), arousal index (p=0.10), and % time <90% saturated (p=0.18).
Conclusions: In this moderate-sized cohort of subjects with OSA verified by polysomnography, women experienced fatigue more commonly than did men even when objective measures of OSA severity were similar. This finding broadens our understanding or how genders manifest symptoms of OSA differently.
Clinical implications: Providers can better capture OSA in women by using the proper questionnaire tool to screen for fatigue, not relying solely on assessments of sleepiness. Future clinical guidelines should incorporate this recommendation to avoid under-recognition of sleep pathology in women.

Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Villines T, Vernalis M. Prediabetes reversal using a novel comprehensive health model. *J Am Coll Cardiol*. 2015;65(10_S):A1414. doi:10.1016/S0735-1097(15)61414-0.

American College of Cardiology (ACC) 64th Annual Scientific Session, San Diego, CA, March 2015. (Poster - recognized with **"Best CV Team"** award.)

Abstract

Introduction: Over half of prediabetics will develop frank diabetes. Prediabetes is a modifiable risk factor for cardiovascular disease (CVD) warranting preventive intervention.
Objective: We examined the impact of a multicomponent intervention on the CVD risk profile of subjects with prediabetes who successfully reversed their disease without emphasizing weight loss.
Methods: Consecutive subjects of the Integrative Cardiac Health Project Registry, a 12-month CVD Risk Reduction Program focusing on four pillars: nutrition, exercise, stress and sleep improvement, completed validated questionnaires and were categorized as prediabetic (glucose ≥ 100 mg/dL and < 140 mg/dL) or reverting prediabetes (glucose < 100 mg/dL). Diabetics were excluded from the analysis. Differences were analyzed using t-test.
Results: Of 508 subjects (56% women, mean age 53 ± 13.5 years, 61% White, 22% Black, 5% Hispanic), 107 (21%) had prediabetes with mean HgA1C 5.9% and mean glucose 108.1 mg/dL. Of prediabetics, 52 (49%) reverted to normal glucose values.

Risk Factor (n=52)	**Baseline**	**6-month**	**p value**
Fasting Glucose (mg/dL)	105.4 ± 6.2	92.4 ± 5.4	< 0.001

Fasting Insulin (uIU/mL)	14.5 ± 10.1	10.4 ± 7.3	0.02
Homeostatic Model Assessment	3.8 ± 2.7	2.4 ± 1.7	0.002
Total Cholesterol (mg/dL)	190.7 ± 41.1	175.1 ± 39.0	0.05
Low Density Lipoprotein (mg/dL)	115.8 ± 36.3	102.5 ± 34.7	0.06
Systolic Blood Pressure (mm Hg)	134.3 ± 15.5	127.9 ± 13.1	0.03
BMI (kg/m^2)	30.0 ± 5.7	29.0 ± 5.8	0.40
Mediterranean Diet Questionnaire (14 points)	6.8 ± 2.4	9.2 ± 2.0	0.002
Aerobic Exercise Time (min/week)	136.4 ± 139.1	192.9 ± 161.7	0.05
Perceived Stress Scale (56 points)	21.9 ± 7.4	18.7 ± 7.0	0.03
Pittsburg Sleep Quality Index (21 points)	7.0 ± 3.4	5.7 ± 3.7	0.08
Fatigue Score (10 points)	4.2 ± 1.9	3.3 ± 2.0	0.03

Conclusion: A comprehensive health program emphasizing combined improvements in nutrition, exercise, stress and sleep may help subjects with prediabetes revert to normal glucose metabolism without substantial changes in BMI. Combatting progression to diabetes with a practical lifestyle intervention lowers CVD risk and improves overall health in this vulnerable population.

This abstract received the following Press Coverage from numerous media sites:
www.physiciansbriefing.com

http://www.acc.org/about-acc/press-releases/2015/03/04/16/49/personalized-health-coaching-helps-reverse-progression-to-diabetes?w_nav=S

http://www.ajmc.com/focus-of-the-week/0315/Personal-Coaching-Halts-Progression-to-Diabetes-in-Some-Patients-Study-Finds

http://www.hcplive.com/conferences/acc-2015/Comprehensive-Health-Model-Provides-Prediabetes-Reversal

http://www.sciencedaily.com/releases/2015/03/150304190114.htm

http://www.endocrinologyadvisor.com/prediabetes-health-program/article/403640/

http://www.medicaldaily.com/prediabetes-patients-reverse-risk-improve-health-when-they-receive-health-coaching-324432

http://www.dailyrx.com/type-2-diabetes-risk-lowered-prediabetes-patients-who-received-health-coaching-diet-exercise-sleep

http://www.pharmacytimes.com/condition-resources/type-2-diabetes/Personalized-Health-Coaching-May-Prevent-Progression-to-Type-2-Diabetes
https://www.facebook.com/permalink.php?story_fbid=809907669062510&id=111902595529691
http://www.acc.org/latest-in-cardiology/articles/2015/03/04/16/32/diabetes-research-and-new-registry-aim-to-improve-outcomes

Kashani M, Eliasson A, Walizer E, Fuller C, Engler R, Villines T, Vernalis M. Early empowerment strategies boost self-efficacy to improve health outcomes. *Circ Cardiovasc Qual Outcomes*. 2015;8(Suppl_2):A331.

(Accepted for presentation - AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2015 Scientific Session – sessions canceled.)
Presented at AHA 2015 Scientific Session, Orlando, FL, November 2015. (Poster)

Abstract

Background: An important mechanism in improving health status in behavioral cardiovascular (CV) self-management programs is patient self-efficacy, or a patient's belief in their ability to make lifestyle changes to reach healthy goals. Little is known on when the optimal time is to incorporate self-efficacy enhancing strategies. We examined the impact of a jumpstart self-efficacy approach in the introductory phase of a multicomponent intervention on CV health outcomes.

Methods: Participants enrolled in a 12-month CV behavioral health intervention, part of a prospective registry, completed validated questionnaires focusing on the four domains of the program: nutrition (Rate-Your-Plate), exercise (minutes of continuous exercise per week), stress (Perceived Stress Scale) and sleep (Pittsburgh Sleep Quality Index); CV-relevant Self-Efficacy Questionnaire. Empowerment strategies comprised a comprehensive risk assessment report with detailed lifestyle recommendations on optimizing risk reduction and a multi-disciplinary educational workshop with interactive healthy food demonstration and stress management instruction. For the remainder of the program, patients received ongoing motivational health-coaching to achieve healthy goals in the four domains. Self-Efficacy Questionnaire was administered at baseline and after the workshop at 6-8 weeks from baseline. All other measures at entry to and at 6-months of the program were assessed with paired t-tests.

Results: Of 88 consecutive completers of the registry, 65 subjects (77%) had a family history of premature CV disease. These high risk subjects (36 men, age 57.4 ± 12.7 years, 42 white, 15 black, 8 other) showed clinically and statistically significant improvements in all four domains as well as improvement in subjective fatigue and self-efficacy for CVD behaviors.

n=65	Self-Efficacy (of 45 points)	Diet (RYP) (of 78 points)	Activity (min/wk)	Stress (PSS) (of 56 points)	Sleep Quality (PSQI) (of 21 points)	Fatigue Visual Scale (of 10 points)
Baseline	34.3 ± 6.9	61.1 ± 8.6	156 ±114	20.7 ± 9.4	7.3 ± 3.6	4.3 ± 2.6
Outcome	40.8 ± 3.6	67.0 ± 5.9	235 ± 174	16.4 ± 8.7	4.6 ± 3.4	2.8 ± 2.2
p value	<0.001	<0.001	0.003	0.008	<0.001	<0.001

Conclusion: A comprehensive CV health intervention emphasizing empowerment strategies early in the sequence of the program improves self-efficacy leading to substantial behavioral improvements in CV health parameters. These findings are highly relevant particularly in high-risk individuals who are vulnerable to CV disease and may have the opportunity to make behavioral lifestyle modifications to lower their risk of overt disease.

Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. High self-efficacy may benefit sleep quality and fatigue. *SLEEP* 2015;38:A295-A296.

Associated Professional Sleep Societies (APSS) Meeting, Seattle, WA, June 2015. (Poster)

Abstract

Introduction: Self-efficacy has been shown to correlate with adherence to positive health outcomes and serves as a pre-condition to promote heart healthy behaviors. Although prior studies associate self-efficacy scores with healthy diet and exercise behaviors, little is known about the association with sleep quality. Given the critical role of healthy sleep behaviors for cardiovascular (CV) disease, we sought to correlate the role of self-efficacy with sleep quality and symptoms of fatigue.

Methods: Consecutive patients (n=89) entering a CV health promotion program completed validated questionnaires, specifically the Pittsburgh Sleep Quality Index (PSQI), fatigue visual analog scale, Rate Your Plate (RYP) diet questionnaire, an exercise question and a CV-relevant Self-Efficacy Questionnaire. In this retrospective analysis, patients were sorted into low (18-35) and high (36-45) score groups for self-efficacy. Groups were compared utilizing t-tests.

Results: Subjects scoring high for self-efficacy (n=44) were not different from those scoring low (n=45) with regard to age (56.2±11.9 vs 54.8±13.1 years, p=0.60), gender (50% vs 51% men, p=0.90), or race (p=0.66). The high self-efficacy group did show better sleep quality (PSQI=6.4±3.0 vs 7.9±4.1, p=0.05), less fatigue (3.5±2.3 vs 4.9±2.5, p=0.007), better RYP score (64.4±7.8 vs 60.0±8.5, p=0.01) and greater exercise minutes (216±131 vs 107±86, p<0.001).

Conclusions: Our findings agree with prior reports that high self-efficacy correlates with healthful diet and exercise habits. We extend this association to include better sleep quality and less fatigue. These findings suggest that efforts to increase self-efficacy may benefit both traditional measures of CV health as well as encompass non-traditional measures, such as sleep health.

Kashani M, Eliasson A, Engler R, Villines T, Vernalis M. Women present with non-traditional precursors of CVD. *J Cardiovasc Nurs* 2016;31(1):10A.

Preventive Cardiovascular Nurses' Association 21st Annual Symposium (PCNA), Anaheim, CA, April 2015. (Poster - **selected for moderated session; received 2nd place ribbon in research competition.**)

Abstract

Background: National guidelines for the evaluation of cardiovascular disease (CVD) risk provide clinicians with global recommendations without specifying differences according to sex.

Hypothesis: We hypothesized that important differences are present in the CVD risk profile of men and women which may point to the need for sex-specific assessment beyond traditional CVD risk scores.

Methods: Subjects presenting to a CVD prevention program underwent comprehensive evaluation for CVD risk including past medical history, family history, smoking exposure, perceived stress assessment with the validated Perceived Stress Scale (PSS), vital signs,

anthropometrics, cardiac-relevant laboratory tests, and calculated Framingham Risk Score (FRS). Differences between men and women were assessed using unpaired t-tests.

Results: Among 300 women and 208 men (mean age 57±12 years), major differences in presentation were:

	FRS	Diagnosed Depression or Anxiety	TChol mg/dL	LDL mg/dL	Non-HDL mg/dL	LP(a) mg/dL	PSS
Women n=300	4.4	32%	197 ±43	115 ±33	134 ±41	82	23
Men n=208	10.9	18%	170 ±39	103 ±35	121 ±39	66	20
p value	<0.001	0.02	<0.001	<0.001	<0.001	0.02	0.001

TChol=total Cholesterol, LDL=low density lipoprotein, HDL=high density lipoprotein, LP(a)=lipoprotein (a)

There were no significant differences in family history of CVD (p= 0.99), smoking exposure (p=0.08), blood pressure (p=0.91 systolic, p=0.10 diastolic), BMI (p=0.66), or laboratory assessment of glucose metabolism (glucose 97.6 for men vs 95.1 for women; p=0.10; insulin 12.7 vs 12.2, p=0.57; HgA1C 5.8 vs 5.9, p=0.17).

Conclusions: Men and women present with CVD risk differently. Mean FRS was significantly lower for women despite worse lipid profiles and the presence of non-traditional precursors for CVD risk such as higher rates of depression/anxiety and perceived stress, all of which may manifest overt disease after menopause. In order to target interventions appropriately, screening approaches for CVD risk should aim to capture sex-specific vulnerabilities for CVD.

The following are key accomplishments during this period of performance:

- Total patient visits: 7896 (includes telephonic coaching calls) of which 150 visits were conducted in 4th quarter.
- ICHP Research Information Management System (RIMS) created and implemented: Completion of 90% of initial objectives.
    - Appropriate SSL certificate obtained and software loaded on production server which will run RIMS application
    - Completed documentation of Project Management Plan, including deliverable schedule, configuration management and quality assurance
    - Project risk analysis completed and mitigation strategies identified and implemented
    - Completed determination of high and mid-level system architecture
    - Implemented application wireframe to include branding, color scheme and general aesthetics
    - Implemented front desk registration page
    - Conversion of selected forms/survey to web-enabled format, including survey scoring
    - Design of patient and provider modules
    - Outline of CHP workflow and process of clinical/research milestones matched with intuitive navigation of application
    - Mechanism established to generate lifestyle prescriptions by compiling CHP data assessment and expertise to serve as potent motivators for participant program adherence to healthy living regimen
    - Workflow of clinical/research milestones aligned with provider process

- Provider specific meetings with IT specialists conducted for clinical feedback
- ICHP staff tested integrity of process and flow
- Front desk process and flow established to dovetail with clinical milestones
- Review and refinement of previous edits completed prior to beta testing
- Beta testing completed
- Front end-survey mechanism built with clinical end testing completed
- Initial Front Desk and clinical team pilot testing successful with additional testing pending.
- CHP Registry and ZENITH protocol unique identification system established
- Expansion of patient modules to include the possibility to receive new alerts from the clinical team
- Training of clinical staff using database process
- Modifications per needs of clinical staff using system
- Configured to launch online surveys to program participants at milestone attainment
- ICHP clinical guidelines incorporated to reflect latest evidence for research protocols
- Full launch for NPs using system for patient visits

- <u>Data Management Plan:</u>
  - Finalized data dictionary for CHP variables
  - Progress continues on merging of data from two previous databases in order to move forward with one dataset for further analysis including 100% quality assurance of data
  - Acquisition of an Outcomes Data Specialist has played a key role in the merging of data
  - Data files de-identified and outcome variables identified for data dictionary
  - Statistical Analysis data fields prepared and normalized for data migration and mining
  - Completion of staff education on statistical methods to analyze data
  - Scientific blueprint established for statistical analyses to identify contribution of ICHP individual interventions

- <u>CHP Quality Improvements/Advancements:</u>
  - Clinical team experts (Clinical Dietitians, Exercise Physiologist and Stress Reduction Specialist) completed requirements for health coach certification
  - All data collection forms for providers and patients revised: toolkit, referral forms, and educational sheets
  - Implemented “integrative synthesis system” for multidisciplinary clinical review team for objective and subjective biometric data review
  - New multidisciplinary initiative created to translate research into practice; Setting the Stage for Success for all domains of the program-anticipating patient needs (pre-emptive and proactive)
  - Implemented multidisciplinary initiative to use patient’s motivational status in an algorithm as a way to prescribe lifestyle practice; allowing for a spectrum of care for patients at different stages of change
  - Created new integrative, combination tracks with multiple aims, i.e. Stress Eating, using stress reduction, sleep expansion and nutrition clinical pathways
  - Began highlighting Family History of premature CVD (high risk factor) to patients (not commonly used in risk scores): 1) Implemented specific assessment and tracking;

2) Developed an Empowerment Dialogue and CVD Risk Acknowledgement Form for Nurse Practitioner use in patients with positive family history of premature CVD, and; 3) Initiated us of Self Efficacy survey to measure the participant's ability to make healthy choices when at high risk for CVD

- Implementation of personalized "Healthy Living Prescriptions" proven successful in patient outcomes.
- Using the AHA as a guide, shifted focus of CHP from disease prevention to health enhancement
- Completed a Quality Improvement project which included a retrospective analysis of 239 patients and a prospective analysis of 58 patients. All outcomes and objectives were met with successful implementation of the ICHP Clinical Decision Support Tool in CHP participants
- Clinical Patient SOP developed and implemented specifically for patients interested in ICHP enrollment who also have other issues: i.e., PTSD, depression, anxiety
- Strategic plan developed to synchronize clinical approach to comprehensive prevention and CV health by standardizing practices of 3 ICHP Nurse Practitioners in interacting with patients and clinical team
- CHP clinical guidelines updated to reflect latest evidence of CV health practice in preparation for initiation of research protocols
- Expansion of food demonstrations to provide patients with practical solutions and ideas to improve the quality and quantity of food in spite of stress eating and diabetes.
- Stress Management CD script in development to include tracks: 1) Rise to a New Day 2) Tension Tamer 3) Power Down for Restful Sleep
- Strategic plan developed to synchronize clinical approach to comprehensive prevention and CV health by standardizing practices of 3 ICHP Nurse Practitioners in interacting with patients and clinical team
- Implemented the inclusion of new CV risk scoring systems (i.e. 10-year risk and lifetime risk) in our clinical model given the guidance from the American College of Cardiology Conference 2014
- ICHP clinical processes refined and SOP(s) created to include patient tracking for numerous protocols revised, including:
  - Standardized Coaching Call Process refinement and implementation to encourage adherence to lifestyle changes in order to maintain gains.
  - Standard protocols for collection of research measurements/examinations: Echocardiograms, Carotid Intima Medial Thickness, Digital Thermal Monitoring, anthropometrics, blood specimen storage, and testing interpretation for study protocols.
  - Guidelines for ZENITH protocol patients matriculating through CHP.
- Staff training completed on above processes/SOPs; competency assessment completed
- Process for streamlining NP process developed and implemented to include comprehensive and updated clinical guidelines
- Efforts to capture ICHP's impact on health improvement as well as CVD risk reduction: quantitative approaches to LIFE Score (Life Impact for Empowerment) in progress
- New ICHP Cookbook (2nd edition) in development
- Process created for ZENITH study subjects to be converted to CHP patients with study

closure
- Creation of engagement tools for patients and their providers:
  - ICHP Healthy Living Toolkit for the Military,
  - Standardized Provider Training Toolkit (Train the Trainers)
  - ICHP Interactive Practical Living Workshop Manual & Slides (2012)
  - ICHP Red, White and Blue Cookbook
  - CD for portable, Stress Reduction & Sleep Enhancement (10-min Tension Tamers)

ICHP Outreach/Marketing Initiatives and Collaborative Efforts:

- All marketing materials updated to reflect transition to WRNMMC Bethesda
- To better reflect ICHP's role in overall warrior health, "Cardiovascular Prevention Program (CPP)" was been changed to "Cardiovascular Health Program (CHP)" -Marketing materials, program forms and protocol revisions are in place reflecting the change
- New marketing strategies created and implemented for future recruitment initiatives including:
  - Hosting of numerous WRNMMC departments in our empowerment workshops (Cardiology, Endocrinology, Integrated Wellness, Executive Medicine, Medical Home, Department of Research Programs, Office of the Surgeon General)
  - News coverage of ICHP, including:
    - WRNMMC's *Journal* with "Heart Health Focus of Program of Excellence at Walter Reed Bethesda" article
    - Fort Gordon's *The Signal* as a program that helps to improve the lives of military beneficiaries
  - Marketing outreach in community settings using presentations that highlight ICHP/CPP as a scalable and relevant program for military and civilian populations: VA Women's Health Clinics in Fairfax and Dumfries, VA.
- Participated in discussions with COL Jeffrey Johnson, Director of Army Wellness at request of TSG Patricia Horoho to discuss ICHP programs and outcomes that may be scalable to complement TSG's Performance Triad. The goal was to determine which aspects of ICHP's program are exportable or could be integrated at other MTFs.
- Development, implementation and continued application of a customized program for Executive Medicine Program to address health of our nation's leaders:
  - Request from Office of the Surgeon General (OTSG) to learn more about ICHP program and its potential in impacting the health and wellness of General Officers.
  - Informational meeting with nutritional food demonstration conducted on 12/15/13 with very positive feedback from OTSG.
  - Creation of customized program to address health of our nation's leaders. Program involved numerous meetings for strategic planning and development of outreach IT software application for collection of health surveys.
  - Multidisciplinary staff expertise used to create personalized plans of care, based on the ICHP model, for spouses of four-star generals and the Army Surgeon General (TSG).
  - Highly successful event (March 4, 2014) executed at the TSG's home at Ft. McNair conducting an interactive Healthy Living Workshop customized for leaders in highly stressed occupations. TSG requested to collaborate further based on the success of is event.

- 2nd Executive Medicine program conducted July 2014 at ICHP with new VIP cohort. BG Clark, WRNMMC Hospital Commander, addressed this cohort and was highly supportive of ICHP efforts.

- Adoption of ICHP's Lifestyle Prescriptions by TSG for BG Clark's Resiliency efforts at WRNMMC. ICHP was honored to provide this service and supported these resiliency efforts at the WRNMMC Prosperity Fairs (October 2014, January 2015, and June 2015). ICHP committed to participating in April 2016 event, but event was subsequently canceled.
- ICHP Clinical team held several coaching workshops for Seton Hill Dietetic Intern.
- Collaborative efforts to expand scientific molecular work with clinical centers of excellence.
- Provided customized educational ICHP handout sent to WRNMMC Heart Failure Clinic.
- ICHP supported the following events/activities through staff participation, educational handouts/presentation, and healthy snacks, cookbooks:
  - Wounded Warrior Prosperity Fair & Welcome Home event
  - February Health Month in collaboration with WRNMMC Cardiology Clinic
  - WRNMMC Prostate Cancer Support Group
- Per request of the U.S Army OTSG, 30 ICHP Cookbooks were delivered for four-star spouse group.

Sub Task #3.1 Continuation of the "Validation of the ICHP Cardiovascular Risk Score" protocol.

Methodology:
Data previously collected on patients enrolled in the Prospective Army Coronary Calcium (PACC) and PACC Rescan projects were reviewed. Specific information was gathered and analyzed to give each patient a CV disease risk score according to a formula developed by the ICHP. This ICHP formula uses the Framingham model of risk prediction and adds historical factors and biochemical markers to produce a novel score predictive of CV disease risk in military beneficiaries. The goal of the study was to validate the utility of this novel ICHP scoring system by comparing the predicted risk with outcomes in this well characterized population. The primary objective of the project was to validate the predictive utility and accuracy of the ICHP CV risk score (or ICHP score). Specifically, the goals: a) to determine if the ICHP score correlates with cross-sectional prevalence of coronary calcium as measured in the PACC project and b) with the development of CHD events such as angina, myocardial infarction, or need for CV intervention such as coronary stenting, angioplasty, or bypass surgery. A third goal: c) to determine the correlation of the ICHP score with coronary calcium progression as measured in the PACC rescan project.

Key Findings/Conclusions:
After statistical analysis of data from the PACC project, ICHP score performed successfully in the linear model with a coefficient of 0.003 (p=0.004), indicating that an increase of one point in ICHP score was associated with increasing CIMT 0.3%. In the logistic model, the odds ratio for the ICHP score was 1.04 (p=0.01), signifying that a one point increase in ICHP score increases odds by 4% of having a top quartile "atherosclerosis score". In conclusion, incorporating novel risk factors such as those proposed in the ICHP score and considering the value of family history

may significantly improve the predictive accuracy of CVD risk assessment and may reveal appropriate targets for therapeutic intervention.

These findings emphasize the need for improved CV disease risk identification in women. Family history and other novel risk factors add predictive value to current risk models and identify potential therapeutic targets.

Status:
This task is complete. Study closure documents submitted to WRNMMC DRP on 16 Dec 14 and approved 30 Dec 14. Closure documents forwarded to USAMRMC HRPO.

Manuscripts Published:

Kashani M, Eliasson A, Vernalis M, Costa L, Terhaar M. Improving assessment of cardiovascular disease risk by using family history: An integrative literature review. *J Cardiovasc Nurs*. 2013;28(6):E18-E27. Review. Epub 2013 Jun 17. doi:10.1097/JCN.0b013e318294b206. ***Cited by 2 PubMed Central articles.***

Manuscript Abstract

Background: Cardiovascular disease (CVD) is the number one killer in the United States. Although the causes of CVD are multifactorial, including genetic and environmental influences, it is largely a preventable disease. The cornerstone of CVD prevention is accuracy in risk prediction to identify patients who will benefit from interventions aimed at reducing risk. Nurse practitioners commonly perform CVD risk assessments and are well positioned to impact preventive therapy. Cardiovascular disease risk scoring systems currently in use substantially underestimate risk in large part because these do not include family history of premature CVD as a high-risk factor.
Purpose: We sought to examine the state of evidence for the use of family history as a predictor in CVD risk stratification.
Conclusions: A comprehensive literature search using the Medical Subject Headings terms of family history of CVD, family history of premature CVD, risk assessment, and risk estimation displayed 416 articles; a review of the titles and subsequent evaluation of the articles eliminated 392 references, leaving 24 for review. By incorporating family history in risk assessment, categorization of CVD risk improves substantially. The evidence demonstrates that family history is an independent contributor to risk appraisal and unequivocally supports its incorporation to improve accuracy in global CVD risk estimation.
Clinical Implications: Underestimation of CVD risk leaves patients and providers misinformed, promoting the ongoing epidemic of chronic disease. Translating this evidence into practice by establishing a clinical algorithm that incorporates family history into risk prediction will standardize CVD risk assessment, improve the identification of high-risk patients, and provide the indicated aggressive care to prevent CVD.

Kashani M, Eliasson A, Vernalis M, Bailey K, Tehaar M. A systematic approach incorporating family history improves identification of cardiovascular disease risk. *J of Cardiovasc Nurs* 2015;30(4):292-297. doi: 10.1097/JCN.0000000000000163. Epub 2014 May 20. ***Cited by 1 PubMed Central article.***

Manuscript Abstract

Background: Although family history (FH) is an independent predictor of cardiovascular disease (CVD) risk, traditional risk scores do not incorporate FH. Nurse practitioners routinely solicit FH but have no mechanism to incorporate the information into risk estimation. Underestimation of risk leaves clinicians misinformed and patients vulnerable to the CVD epidemic.

Objective: We examined a systematic approach incorporating FH in CVD risk assessment, validating risk reclassification using carotid intima-media thickness (CIMT), a surrogate measure of atherosclerosis.

Methods: Of 413 consecutive patients prospectively enrolled in the Integrative Cardiac Health Project Registry, a subgroup of 239 was low or intermediate risk by the Framingham Risk Score. A systematic approach for the assessment of FH was applied to this subgroup of the registry. A positive FH for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, conferred reclassification to high risk. Reclassification was validated with CIMT results.

Results: Chart audits revealed adherence to the systematic approach for FH assessment in 100% of cases. This systematic approach identified 115 of 239 (48%) patients as high risk because of positive FH. Of the reclassified patients, 75% had evidence of subclinical atherosclerosis by CIMT versus 55% in the patients not reclassified, P = 0.001. Logistic regression identified positive FH for premature CVD (odds ratio, 2.6; P = 0.001) among all variables, as the most significant predictor of abnormal CIMT, thus increasing risk for CVD.

Conclusions: The Integrative Cardiac Health Project systematic approach incorporating FH into risk stratification enhances CVD risk assessment by identifying previously unrecognized high-risk patients, reduces variability in practice, and appropriately targets more stringent therapeutic goals for prevention.

Abstracts Presented:

Kashani M, Eliasson A, Bailey K, Vernalis M, Terhaar M. Systematic inquiry of family history improves cardiovascular risk assessment. *Circ Cardiovasc Qual Outcomes.* 2013;6(:A314.

AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2013 Scientific Session, Baltimore, MD, May 2013. (Poster)

Abstract

Background: Numerous research publications substantiate the value of family history of premature cardiovascular disease (CVD) as an independently predictive risk factor for CVD. However, many traditional CVD risk assessment tools do not incorporate family history and therefore underestimate true risk. Risk assessment is the cornerstone of determining therapeutic goals and preventive intervention.

Objective: We sought to implement and validate a clinical decision support tool that incorporates family history as a high-risk factor to guide risk stratification in a population identified as low and intermediate risk by Framingham Risk Score (FRS).
Methods: In a retrospective analysis of patients entering our CVD prevention program, the clinical decision support tool was applied. The clinical decision support tool defines family history of premature CVD as having a parent and/or sibling with a CVD event before the age of 55 in men and 65 in women. Patients entering our CVD prevention program have a comprehensive risk assessment including measurement of blood pressure, lipids, glucose metabolism and Carotid Intima Media Thickness (CIMT).
Results: Of 394 patients entering our program, 239 (61%) were scored as low or intermediate risk by FRS. These patients had a mean age of 49 years (range 20-76), 60% women, 52% Caucasian, 37% Black, 5% Hispanic and 6% other. The decision support tool identified 114 (48%) of these patients as high-risk for CVD as a result of the inclusion of family history of premature CVD. In these patients, 72% had dyslipidemia, 35% hypertension, 20% prediabetes and 65% had early atherosclerotic disease by CIMT.
Conclusions: In our population, almost half of the patients previously determined as low or intermediate risk were reclassified as high-risk for CVD. This reclassification is underscored and validated in view of both the high frequency of metabolic abnormalities revealed and the atherosclerotic burden seen by CIMT. These findings are highly relevant particularly in middle-aged women who are frequently more vulnerable to overt heart disease and require improved risk assessment for aggressive treatment goals to enhance prevention.

Bailey K, Kashani M, Eliasson A, Vernalis M. Low self-efficacy correlates with increased cardiovascular disease risk. *Circ Cardiovasc Qual Outcomes.* 2013;6(A262.

AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2013 Scientific Session, Baltimore, MD, May 2013. (Poster)

Abstract

Background: Cardiovascular disease (CVD) is largely a preventable illness contingent upon the ability of patients to adhere to healthy lifestyle choices. Self-efficacy is a strong predictor of a patient's ability to adopt healthy lifestyle change. We sought to examine correlations between self-efficacy and CVD risk.
Methods: The Integrative Cardiac Health Project is an interdisciplinary CVD prevention program that seeks to improve modifiable CVD risk factors among military healthcare beneficiaries. Participants are provided comprehensive assessments and consultations within the disciplines of exercise, nutrition, stress and sleep to identify and modify CVD risk. Upon initiation of the program, participants complete a battery of assessments including anthropomorphic measurements, fasting laboratory studies and validated surveys for self-efficacy and sleep. Sleep surveys include the Berlin Questionnaire for sleep apnea, Pittsburgh Sleep Quality Index (PSQI) for global assessment of sleep quality, and the visual analog fatigue score. In this retrospective analysis, patients were sorted into low and high self-efficacy groups using the midpoint of the range of self-efficacy scores. Patients with low (10 to <16.25 points) and high (16.25 to 22.5 points) self-efficacy scores were compared utilizing t-tests.

Results: Of 71 participants (mean age 52 yrs, 46% men), 25 (35.2%) had low self-efficacy scores. Subjects with low self-efficacy scores demonstrated numerous CVD risk factors (See table).

Score	DBP (mmHg)	BMI (kg/m^2)	WC (cm)	HgA1C (%)	Insulin (uIU/mL)	CRP (mg/dL)	Berlin (%)	PSQI (points)	Fatigue (points)	Sleep Time (hrs)
Low Self-Efficacy	82.3	34.0	108.0	5.9	18.0	0.56	0.88	9.5	5.24	5.77
High Self-Efficacy	77.7	27.8	94.4	5.6	10.6	0.23	0.57	7.5	4.09	6.47
p-value	0.058	<.001	0.001	0.029	0.007	0.016	0.015	0.013	0.037	0.021

Conclusions: Self-efficacy and CVD risk profiles appear to be inversely related. To lower CVD risk and improve adherence to healthy lifestyle change, strategies to improve patient self-efficacy must be a primary consideration.

The following are key accomplishments during this period of performance:

- The ICHP manuscript entitled, *A Systematic Approach Incorporating Family History Improves Identification of Cardiovascular Disease Risk*, was included as evidence in new AHA/ACC Clinical Guidelines for CVD Risk Assessment[5].
- This above manuscript (Doctoral Dissertation) was included in the following textbook for doctoral students with copyright for international dissemination:
  White K, Dudley-Brown S, and Terhaar, M. (2016). Translation of Evidence into Nursing and Health Care, Second Edition. Chapter 19: Population Health (Exemplar: Kashani, M).
- ICHP Clinical Decision Support Tool implemented and actively utilized in clinical encounters to improve CVD risk classification.

Sub Task #3.2: Initiate the "ZENITH (randomiZed Evaluation of a Novel comprehensIve prevention program on aTHerosclerosis progression) Trial" at WRNMMC ICHP. This study represents collaboration between WRNMMC Cardiology Services, ICHP and Windber Research Institute (WRI).

Methodology:

The purpose of this one-year, prospective, randomized, controlled, interventional trial is to investigate the impact of ICHP-CPP on vascular health, atherosclerosis progression and left-ventricular relaxation (diastolic function) among patients with increased lifetime CVD risk, but low short term coronary heart disease (CHD) risk (according to the Framingham Risk Score, FRS) as compared to receiving usual care (UC). Up to 170 male and female patients between 18-50 years of age with low (<10%) 10-year FRS for CHD but estimated lifetime risk (to age 95 years) of coronary death or myocardial infarction (MI) of ≥ 39% without clinically manifest CVD [MI, coronary or peripheral arterial revascularization, obstructive coronary artery disease (CAD), heart failure or cerebrovascular event] will be randomized to participation in the currently ongoing ICHP-CPP or to UC. The primary endpoint is between-group differences in the change in vascular endothelial function as measured using DTM, as reported as adjusted. Secondary endpoints are changes in measures for CIMT, cardiac diastolic function, lifetime CHD risk scores, and the ICHP CV Risk Score. It is hypothesized that patients with low-short term (Framingham 10-year CHD risk score) but high lifetime estimated risk for coronary death or MI who participate in the ICHP-CPP will improve vascular health and reduce atherosclerosis

progression when compared to those receiving usual care. Blood storage and analysis of biomarkers will take place at Windber Research Institute.

Status:
This protocol was submitted to WRNMMC DRP 10 May 2012. Administrative revisions completed on 25 Jun 2012. The WRNMMC protocol received WRNMMC IRB approval on 13 Dec 2012 and USAMRMC HRPO approval on 10 Jun 2013.

The Windber Medical Center (WMC) IRB approved the WRI protocol on 29 May 2013 and forwarded to USAMRMC HRPO for review. HRPO asked for changed in WMC determination to "no human use" and change was subsequently sent to HRPO on 9 Sep 2013. Final HRPO approval for the WRI protocol was received on 13 Oct 2013. When the WMC IRB disbanded, the WRI protocol was reviewed by the Chesapeake IRB and received approval on 6 Mar 2014 (No Human Use Determination).

Several amendments were approved for the WRNMMC protocol. Following is a summary of those minor changes:

1) Updates to recruitment materials, case report forms, change in temporary serum storage location at WRNMMC and several edits to investigator information; approved 7 January 2014.
2) Inclusion of a Self-Efficacy Questionnaire; 11 April 2014
3) Expansion of recruitment efforts to include public spaces at the WRNMMC and NSA-Bethesda, addition of AI and several other minor edits; 28 December 2014.
4) Enhancement of the recruitment plan to include study visibility in WRNMMC/Naval Support Agency social media sites and the pre-identification of potentially eligible participants through scheduled Cardiology Clinic patients; approved 22 July 2015.

A statistical support contract was executed for this protocol. Under this contract, ICHP received support to review the protocol methods as well as case report forms. Additionally, a preliminary data management plan (DMP) was established. Planning for study execution was active during this period of performance. Planning for study execution was very active during this period of performance which included the following: establish study procedures, develop recruitment plan, develop randomization process/scheme, procuring equipment/supplies, training research staff, development of study checklists/logs, develop preliminary database, and establish processes for collection/storage of blood samples.

Execution of the study was delayed by several technology issues: 1) Loss of a trained ultrasound technician; 2) Carotid ultrasound equipment failure and significant delays in the procurement of a replacement machine; 3) Hiring/training of Cardiovascular Ultrasound Technician; 4) Difficulty in performance of echocardiogram software on local computer followed by need to upgrade system, and; 5) Inability to transfer and locally archive echocardiogram research images. These issues took over a year to resolve and recruitment commenced early July 2014.

Despite an enhancement in recruitment efforts, including a review of hundreds of Cardiology clinic patient records, enrollment was difficult. Since initiation of study recruitment in July 2014, a total of 74 potential participants were screened, 19 enrolled and provided informed consent, 6

screened out after enrollment (5 had lifetime risk <39%, 1 was participating in a drug study), 14 randomized (7 – ICHP group; 7 – controls) and 2 withdraw (1 for unplanned relocation; 1 no longer wanted to participate). There was 1 study completer (control group).

Upon review of the enrollment rate and assessment of potential enrollment to meet the study timeline, a decision was made to stop recruitment and close the study. Study Closure documents were forwarded to WRNMMC DRP on 28 January 2016 and approved 10 February 2016. Closure documents forwarded to HJF on 12 Feb 2016 for submission to HRPO.

Findings:
No data analysis completed.

Adverse Events:
One adverse event was reported to the WRNMMC IRB during the length of this study.

Sub Task #3.3: Initiate the "Cardiovascular Health Program (CHP) Registry for the Integrative Cardiac Health Project" protocol.

Methodology:
The purpose of this study is to establish a registry to enable research on patients at risk for cardiovascular disease (CVD). All clinically derived patient-related data for subjects participating in the WRNMMC CHP will be entered into a single, secure database. At periodical intervals, assessment of the registry database will allow queries to define the impact of an integrative lifestyle change program on CVD risk over time. The ICHP Registry will utilize the ICHP database which documents demographics, responses to validated lifestyle habits questionnaires regarding exercise, diet, stress and sleep, physical examination and anthropometrics, laboratory test results, imaging, actigraphic data, clinical recommendations and consultations, participant management, and participant visits.

Patients will be offered enrollment into this study at the time of presentation if they are military health care beneficiaries and are at least 18 years of age. All participants, regardless of enrollment in the study, will receive the usual standard of care by their health care providers. Collection of medical information on ICHP subjects is accomplished through interview of patients as well as through review of medical information from other facilities providing care. Clinical data collection occurs at baseline and at the conclusion of the intervention, typically at 6 months. Additional follow up for support of the patient's gains and additional data collection occur at 12 months and annually for up to 5 years. The research component of this study will involve the analysis of clinical data collected at these intervals.

The ICHP clinical database can be queried at a single sitting with removal of all personally identifying information to perform assessments of prevalence of risks, associations of behaviors and risks, and the success of various interventions over time. Such queries take minutes to perform and can be accomplished with minimal risk to individual privacy. There is no need to maintain any linkage data as the information is harvested at a single sitting from one database requiring no marriage with external data sets.

Status:
Protocol was submitted to WRNMMC DRP on 13 December 2011, received Scientific Review approval on 28 September 2012 and final WRNMMC IRB approval on 27 March 2013. USAMRMC HRPO requested revisions to the consent form and approved by WRNMMC IRB on 15 July 2013. Final USAMRMC HRPO approval was received 13 November 2013. A decision was made to delay recruitment until initial ICHP database testing completed in November 2014.
Clinical Trial Registration—URL: www.ClinicalTrials.gov. Unique Identifier: NCT01975181.

The following amendments have been submitted during this reporting period:
1) Updates to the ICHP CHP lifestyle questionnaire and survey packet.
2) Updates to current investigators' contact information, addition of AI and inclusion of the RAND 36-Item Health Survey and Self-Efficacy Questionnaire as part of the standard ICHP Cardiovascular Prevention Program (CPP) survey packet.
3) Change of WRNMMC PI, Cardiovascular Prevention Program (CPP) was changed to Cardiovascular Health Program (CHP) to fully reflect the comprehensive nature of the ICHP program, numerous changes/edits made to reflect current WRNMMC template language, inclusion of all non-active patients (up to 1000) who participated in CHP between 2005 and 2014 (waiver to consent) into registry, and prospective enrollment of all active CHP participants at any visit during their CHP participation.
5) Addition of an AI.

A total of 191 active ICHP-CHP participants have been consented in a prospective fashion since December 2014. An annual Continuing Review was approved by WRNMMC DRP on 17 March 2016; approval sent to HRPO for acknowledgement. There have been no adverse events reported for this study. Data analysis and publication of findings are ongoing.

Manuscript Published:

Kashani M, Eliasson AH, Walizer EM, Fuller CE, Engler RJ, Villines TC, Vernalis MN. Early empowerment strategies boost self-efficacy to improve cardiovascular health behaviors. *Glob J Health Sci* 2016 Feb 2;8(9):55119. doi: 10.5539/ghis.v8n9p322.

Manuscript Abstract

Background: Self-efficacy, defined as confidence in the ability to carry out behavior to achieve a desired goal, is considered to be a prerequisite for behavior change. Self-efficacy correlates with cardiovascular health although optimal timing to incorporate self-efficacy strategies is not well established. We sought to study the effect of an empowerment approach implemented in the introductory phase of a multicomponent lifestyle intervention on cardiovascular health outcomes.
Design: Prospective intervention cohort study
Methods: Patients in the Integrative Cardiac Health Project Registry, a prospective lifestyle change program for the prevention of cardiovascular disease were analyzed for behavioral changes by survey, at baseline and one year, in the domains of nutrition, exercise, stress management and sleep. Self-efficacy questionnaires were administered at baseline and after the empowerment intervention, at 8 weeks.

Results: Of 119 consecutive registry completers, 60 comprised a high self-efficacy group (scoring at or above the median of 36 points) and 59 the low self-efficacy group (scoring below median). Self-efficacy scores increased irrespective of baseline self-efficacy but the largest gains in self-efficacy occurred in patients who ranked in the lower half for self-efficacy at baseline. This lower self-efficacy group demonstrated behavioral gains that erased differences between the high and low self-efficacy groups.
Conclusions: A boost to self-efficacy early in a lifestyle intervention program produces significant improvements in behavioral outcomes. Employing empowerment in an early phase may be a critical strategy to improve self-efficacy and lower risk in individuals vulnerable to cardiovascular disease.

Abstracts Presented/Accepted:

Engler R, Kashani M, Eliasson A, Walizer E, Fuller C, Villines T, Vernalis M. Blood pressure elevations below hypertension threshold linked to insulin resistance and dyslipidemia: An under-recognized cardiovascular disease risk phenotype. Military Health System Research (MHSRS) Symposium 2016, Kissimmee, FL, August 2016. (Accepted for poster presentation)

Abstract

Background: Cardiovascular disease (CVD) morbidity/mortality risk has been directly correlated to blood pressure (BP) levels with lower levels, even below "normal ranges", associated with reduced CVD risk. Yet current clinical guidelines only address treatment for frank hypertension (equal/over 140/90 mmHg).There is increasing interest in earlier and more precise identification of CVD risk particularly for enhanced lifestyle management interventions to prevent disease and reduce lifetime risks. Metabolic dysfunction characterized by insulin resistance predicts future risk for type 2 diabetes mellitus (T2DM) and is potentially reversible. The homeostatic model assessment (HOMA) is a calculated value that reflects hepatic insulin resistance (IR). Early preclinical diabetes with increased IR affects a large population (86 million Americans) and has gone largely unrecognized. Improving the precision of CVD risk assessments in order to guide earlier more effective intervention strategies can reduce the burden of future CVD risk complications.
Methods: Between July 2005 and July 2015, consecutive subjects entering the Integrative Cardiac Health Project (ICHP) Registry (a12-month prospective CVD Risk Reduction Program) were assessed for BP category and prevalence of metabolic risk factors by measuring anthropometrics and CVD-relevant laboratory parameters including insulin resistance by HOMA. HOMA values greater than 2.0 to 3.0 are associated with increased CVD risk in adult populations. BP was categorized as not elevated (less than 120/80), modestly elevated (between 120/80 and 140/90, also described as prehypertension) and hypertensive (equal/over 140/90). Comparisons were made between subjects with no BP elevation, modest BP elevation and hypertensives for differences in CVD risk factors using t-test analysis. These BP groups were compared for the following CVD risk parameters: fasting glucose (Gluc), hemoglobin A1C (HgbA1C), HOMA-IR, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), body mass index (BMI), and waist circumference (WC).
Results: Of 352 subjects (56% women, mean age 53 ± 13.5years, 61% white, 22% black, 5% Hispanic), 114 (32%) had no elevation in BP,154 (44%) had modest elevation in BP and 84 (24%) were hypertensive. There were no differences between the hypertensive group and those

with modest elevation in BP. There were significant differences in means (+/- SD: standard deviation) between those without elevated BP and the group with modestly elevated BP for the variables detailed: Gluc [93.9(16.7) vs 100.6(14.9), p=0.001]; HgbA1C [5.5(0.06) vs 5.7(0.06), p=0.02]; HOMA [2.89(2.6) vs 3.75(3.8), p=0.01]; HDL[60.4(17.0) vs 55.2(13.6), p=0.009]; TG [97.6(50.7) vs 115.7(66.1), p=0.012]; BMI[28.2(5.8) vs 30.5(5.5), p=0.0006]; WC [94.3(15.1) vs 102.8(14.1), p=0.0001]. There were no significant differences in LDL levels [108.5(28.7) vs 115.0(38.0), p=0.12].
Conclusion: We demonstrate that among subjects with pre-hypertension, there is a significant prevalence of insulin resistance, dyslipidemia and obesity. Modest elevations in BP may identify subjects with metabolic syndrome who may benefit from enhanced preventive interventions. Given the many military service associated confounders exacerbate CVD risk, there is a need for improved earlier diagnosis of clinical conditions that can and should be addressed to maintain optimum health of the force.

Citation: Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Targeted behavioral interventions improve disturbed sleep. *Sleep* 2016; 39:A397. (citation available prior to 31 May 2016)

APSS 2016 Meeting, Denver, CO, June 2016. (Accepted for poster)

Abstract

Introduction: Sleep is an established risk factor for cardiovascular disease (CVD). CVD prevention programs are an ideal setting to assess patients for disturbed sleep. For our CVD prevention program, we report the frequency of disturbed sleep and improvement of important outcomes.
Methods: At baseline, patients completed validated questionnaires: Berlin Questionnaire for sleep apnea, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and Stanford Fatigue Scale. After CVD risk assessment by a nurse practitioner, patients attended a healthy lifestyle workshop with didactics on healthy sleep practices, experiential stress reduction, and food demonstration. All patients received personalized lifestyle prescriptions. Patients with abnormal sleep surveys received customized sleep recommendations. Over 12 months, patients were coached on diet, exercise, and stress management. Validated surveys were repeated at graduation. Means and standard deviations provide descriptive statistics. Two sample t-tests measure statistical significance for changes from baseline to graduation.
Results: Of 455 consecutive program completers, 59% women, there were 61% white, 31% black, 4% Hispanic, 2% Asian, 2% other. Fifty-one patients (11%) entered the program with previously diagnosed sleep apnea. Screening for sleep apnea was positive in 217 more patients (48%) consequently referred for polysomnography. Of the remaining 187 patients (41%), 68% had poor sleep quality (mean PSQI 7.8±2.8, normal sleeper <5 points), mean sleep duration 6.6±1.2 hours, ESS 7.3±4.4, and fatigue score 3.4±2.2. Of patients with poor sleep quality (68%), PSQI improved 2.2 points, p<0.001; 54% improved sleep duration 30 minutes, p=0.007; 71% improved ESS 3 points, p<0.001, and 58% improved fatigue 1.2 points, p<0.001.
Conclusions: Our CVD prevention program provides an opportune mechanism to identify sleep disturbances. Nearly 2/3 of our population screens positive for sleep apnea and a majority of the remainder experience poor sleep quality and duration. Targeted interventions for improved sleep are effective and support CVD risk modification.

Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. *Ann Behav Med* 2016 Mar;50 Suppl 1:S124. doi: 10.1007/s12160-015-9766-4

Society of Behavior Medicine (SBM) 37th Annual Meeting & Scientific Session, Washington DC, March 2016. (Poster)

Abstract

Background: Self-efficacy, or confidence in the ability to carry out behavior to achieve a desired goal, is considered to be a prerequisite to behavior change. Prior research has shown that efforts to improve self-efficacy correlate with greater adherence to dietary guidance and exercise prescriptions or both combined. However, the role of self-efficacy for stress management and sleep improvement has not been well studied. We sought to examine the effect of empowerment strategies for self-efficacy on a multicomponent lifestyle intervention focusing on four behaviors: diet, exercise, stress management and sleep.
Methods: Patients in the Integrative Cardiac Health Project Registry, a prospective lifestyle change program for the prevention of cardiovascular disease were analyzed for behavioral changes using validated surveys, at baseline and one year, in the domains of nutrition, exercise, stress and sleep. Self-efficacy questionnaires (9 questions, maximum possible score 9 x 5=45 points) were administered at baseline and after the empowerment intervention, at 8 weeks. Data from baseline and one year were compared with t-tests.
Results: Of 119 consecutive program completers, 98 (82%) showed improvements in self-efficacy. Data sets were normally distributed. For all patients, self-efficacy scores increased a mean of 5.8 ± 5.1 points. There were consequent improvements in dietary adherence (61.7 ± 8.3 to 67.1 ± 6.0, R=5.8, $p<0.001$), exercise minutes (156 ± 125 to 220 ± 163, R=3.4, $p<0.001$), stress scores (20.1 ± 9.1 to 17.2 ± 8.6, R=2.6, $p=0.01$), sleep quality (7.1 ± 3.9 to 4.7 ± 3.5, R=4.8, $p<0.001$) and fatigue (4.3 ± 2.5 to 3.0 ± 2.2, R=4.2, $p<0.001$). These findings remained statistically significant after Bonferroni correction.
Conclusions: A boost to self-efficacy in a lifestyle intervention program produces substantial improvements in behavioral outcomes. This study validates prior reports that efforts to improve self-efficacy improves adherence to diet and exercise regimens and extends the finding to improvements in stress management and sleep.

Sub Task #3.4 Collaboration on "Assessing Risk Factors for Cardiovascular Disease in Individuals with Traumatic Amputations" protocol (PI: Alison Pruziner), DPT, ATC, WRNMMC Dept of Rehab). This study represents a collaboration involving the WRNMMC Department of Rehabilitation, WRNMMC Department of Nutrition Services, WRNMMC ICHP, Windber Research Institute, and Myriad RBM, Inc.

Methodology:
The objective of this comparative cohort study is to assess presence of known risk factors for CVD in individuals with traumatic amputations. Up to 405 participants will be enrolled and divided into three groups: no injury, traumatic orthopedic injury with amputation, traumatic orthopedic without amputation. Data will be collected at two time points, at time of consent and at a 5-year follow-up visit, and will include demographic (including diagnosis of hypertension, hyperlipidemia or diabetes mellitus) and family history, anthropometric (height, weight, waist circumference, hip circumference

and body composition), biochemical (lipids, fasting blood sugar, hemoglobin A_{1c}, fasting insulin, ultra-sensitive C - reactive protein, lipoprotein (a), thyroid stimulating hormone, vitamin D, and fibrin D-dimer), blood pressure, heart rate, pulse pressure, EKG, carotid intima-medial thickness (CIMT) study, stress and sleep surveys, diet (fruit and vegetable intake, total fat and saturated fat intake), smoking history and activity measures. CVD risk will be estimated using the Integrated Cardiac Health Project (ICHP) risk assessment and the National Heart Lung and Blood Institute (NHLBI) 10-year risk estimate. It is hypothesize that: 1) Individuals with traumatic amputations (A) will have higher levels of factors that increase risk (anthropometry, biochemical markers, blood pressure, pulse pressure, CIMT, stress, poor sleep habits, saturated fat intake, smoking) and lower levels of factors that decrease risk (fruit and vegetable intake and activity) for CVD when compared to individuals without orthopedic injuries (N), and that this risk will continue to increase over the 5-year follow-up; 2) Individuals with traumatic amputations (A) will also have the same increased risk factors, as stated above, when compared to individuals with traumatic orthopedic injuries that did not result in amputation (O), and again this risk will continue to increase over the 5-year follow-up, and; 3) There will be no difference in presence of risk factors between individuals with (O) and without orthopedic injuries (N), that did not result in amputation.

Status:
This study received WRNMMC IRB approval on 23 Feb 2012 and enrollment/data collection commenced in March 2012. ICHP personnel involvement (collection of EKG, CIMT, training on questionnaire scoring guidelines) began upon MRMC HRPO approval in August 2012.

During this period of performance, several amendments have been submitted and approved:
1) Inclusion of extramural funding source.
2) Updates to Associate Investigators and several Case Report Forms.
3) Change of PI and collection of additional blood for molecular analysis (PAXGene and multi-analyte panel (MAP) samples.
4) PI last name change and change in processing/temporary storage location of MAP/PAXGene blood samples.
Training was also conducted in the WRNMMC Biomolecular Research Lab in collaboration with BRL staff and WRI personnel on procession/storage of the MAP and PAXGene blood samples.

Total study enrollment=54 (24 controls, 26 amputees, 4 limb salvage). Study enrollment has been low primary due to limited recruitment support for PI. The PI is currently seeking avenues for funding personnel position to enhance recruitment/data collection. ICHP continues to support collection of CIMT, EKG and ICHP questionnaires for risk assessment. MAP sample/PAXGene sample collection/storage began in June 2015. There are currently 2 subjects with stored samples. Annual continuing review was approved by WRNMMC DRP on 14 Dec 2015 and forwarded to HRPO for acknowledgement.

Preliminary Results: No data reported at this time.

Adverse Events: None.

Sub Task #3.5 Collaboration on "Integrative Cardiac Health Project Cognitive Behavior Therapy for Insomnia (ICHP CBT-I)" Pilot Study. This study was designed and submitted by MAJ Catherine Ware, a USUHS doctoral student, in collaboration with WRNMMC ICHP. Phase 1 of this study, as described below, was commenced during this period of performance.

Methodology:
The purpose of this study is to investigate the feasibility, acceptability, and effectiveness of adding Cognitive-Behavioral Therapy for Insomnia (CBT-I) to the standard care received at the Integrative Cardiac Health Project (ICHP). The study addresses an important gap in cardiovascular prevention research. Previous research has shown associations between insomnia and increased cardiovascular risk, but no trials have tested the feasibility and acceptability of an insomnia intervention within this population. Additionally, the impact of CBT-I in a cardiovascular risk prevention program is unknown.

This two-arm, randomized controlled interventional trial is conducted among patients with insomnia currently enrolled in the ICHP cardiovascular risk prevention program. This study will be completed in two phases: Phase I—feasibility and acceptability, and Phase II—effectiveness of intervention.

To conduct both phases of the study, up to 64 total male and female patients enrolled in ICHP who meet criteria for insomnia are being recruited. Insomnia is defined according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), and severity is measured with the Insomnia Severity Index. Patients who meet inclusion and exclusion criteria for this study and consent to participate are randomized to one of two conditions: (1) ICHP, or (2) ICHP + CBT-I treatment. CBT-I treatment will consist of four in-person appointments and two telephone appointments. Each in-person session will be approximately 1 hour, and each phone session will be approximately 30 minutes.

Phase I: The first phase examines the feasibility and acceptability of CBT-I within the existing ICHP cardiovascular risk prevention program.

It is hypothesized that recruitment rate, consent rate, treatment completion rate, and compliance with questionnaires will be large enough to facilitate an adequately powered study. It is also hypothesized that participants will indicate acceptability by answers to self-report measures such as the Insomnia Treatment Acceptability Scale, Insomnia Treatment Evaluation Measure-Revised, and a qualitative interview at completion of treatment. Finally, it is hypothesized that ICHP program resources and management processes will be adequate to conduct Phase II (the larger trial). These resources include necessary elements such as physical space to conduct the intervention and adequate equipment. Management processes include investigator and staff administrative capacity to accommodate the intervention. Examples include participant scheduling, data collection, file management, and data entry. Qualitative data is being collected through observation and tracking of logistical challenges throughout Phase I of this study.

Phase II: The second phase will investigate the effectiveness of ICHP compared to ICHP + CBT-I on sleep outcomes, fatigue, sleepiness, depression, stress, and sleep-related quality of life. It will

also explore the impact of sleep improvement on change in secondary cardiovascular risk factors such as high blood pressure and elevated cholesterol.

It is hypothesized that participants in the CBT-I treatment group will demonstrate a greater improvement in traditional sleep measures compared to the control group as measured by changes in: sleep onset latency (SOL), wake after sleep onset (WASO), total sleep time (TST), and sleep efficiency (SE) as measured by sleep diary, actigraphy, and the Pittsburgh Sleep Quality Index (PSQI) score. Further, it is hypothesized that participants in the CBT-I treatment group will have less fatigue, lower severity of insomnia symptoms, fewer depressive symptoms, lower perceived stress, lower daytime sleepiness, and higher sleep-related quality of life as measured by the Functional Outcomes of Sleep Questionnaire at post-treatment than the ICHP only group.

Finally, it is hypothesized that participants in the CBT-I treatment group will show more improvement in secondary cardiovascular risk factors than the ICHP group at post-treatment, 2 month follow-up, and 4 month follow-up. Secondary cardiovascular risk factors hypothesized to show improvement include: blood pressure, cholesterol, body mass index, and waist circumference. One additional risk factor hypothesized to show improvement includes C-reactive protein.

Status:
This study received WRNMMC IRB approval on 8 June 2015. A USUHS concurrence letter was received 30 June 2015. USAMRMC HRPO approval was received 7 July 2015. Recruitment and screening for Phase 1 began in September 2015.
Clinical Trial Registration--(URL: www.ClinicalTrials.gov. Unique Identifier: NCT02779023.)

The first participant for Phase I was enrolled in January 2016. 23 total participants were screened in order to enroll 5 total participants in the study. Eighteen (18) total were excluded for a positive sleep apnea screening and 9 because they did not meet required severity of sleep disturbance at screening. Five (5) total participants have provided informed consent. One participant completed informed consent but was not eligible to continue in the study after further screening. Of the 4 randomized, 2 were randomized to the control group (ICHP only) and 2 were randomized to the treatment group (ICHP + CBT-I).

An amendment to the inclusion and exclusion criteria was approved for Phase I in January 2016 to allow the inclusion of participants screening positive for sleep apnea. This amendment increased the enrollment rate. Another amendment is planned to request extending this adjusted inclusion and exclusion criteria to Phase II.

At the time of this writing, one participant has completed all of the CBT-I appointments and is waiting to complete follow-up assessments only. There have been no adverse events.

The annual Continuing Review was approved on May 23, 2016.

Preliminary Results:
Preliminary results suggest that conducting the CBT-I study within ICHP is both feasible and acceptable to participants and research staff members. This is based on the recruitment rate (with

amended criteria), consent rate (80% with preliminary numbers), treatment completion rate, and availability of ICHP program resources to allocate toward this study. Further, initial participant responses on self-report measures of acceptability as well as an exit interview at the end of the study suggest sufficient feasibility and acceptability.

Adverse Events: None.

Task #4: Follow-up data analysis and publications for the following protocols at Windber Research Institute (WRI): 1) Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal and the Sub-Study for Subjects in the Dr. Dean Ornish Program, and; 2) Cardiovascular Risk Assessment and Prevention Program through the Cardiovascular Risk Clinic (CRC).

Status:
Although enrollment in these programs is completed, data collection was finalized and data analysis continued on biochemical markers, gene expression and SNP data and PET/CT imaging studies. Results were also prepared for publication.

Ornish Program Status:
Enrollment into the Dr. Dean Ornish Program is closed and all active participants completed their participation in the study. Continuing review of the protocol was approved by the Chesapeake IRB on 11 Jun 2014 (99-00).

Subject Enrollment and Demographics:
The Ornish program is closed to enrollment and all active subjects have completed the program. Subject enrollment was 422 participants including 25 cohorts and 4 retreats. 339 participants graduated from the program and 83 participants discontinued participation (20% dropout rate). Demographic characteristics of participants were: average age of 66.1 years, 53% female, 33% veterans or the spouse of a veteran, and 41% had diagnosed coronary heart disease.

Outcome Data:
Participants in the Dr. Dean Ornish Program at Windber Medical Center achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period. Measures of obesity including weight and BMI declined ~7%, levels of total cholesterol were reduced by nearly 13%, blood pressure dropped ~9%, measures of physical fitness increased more than 26%, and levels of depression decreased approximately 47%. These data demonstrate that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Over the course of one year, weight and BMI decreased ~9%, diastolic blood pressure decreased ~7%, measures of physical fitness increased 25%, and levels of depression decreased nearly 50%.

Global Profiling Status:
Enrollment to the global profiling study is closed and all active participants have completed their participation in the study. Enrollment in the sub-study was closed as of July 27, 2007. Data analysis is ongoing. Continuing review of the protocol was approved by the Chesapeake IRB on 14 Jan 2015 (03-03).

Subject Enrollment and Demographics:
Subject enrollment was 374. There were 166 participants taking part in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study. Demographic characteristics of the control group were: average age of 63.7 years, 51% were female, 29% were veterans or the spouse of a veteran, and 34% had diagnosed CHD.

Data:
Lipoproteins – LipoScience devised the new LP3 analysis process to better account for the full diversity of plasma lipoproteins that span a continuum of particle diameters and a Lipoprotein Insulin Resistance Score (LP-IR), which is significantly associated with insulin resistance.

Abstract Presented:

Ellsworth DL, Mamula KA, Blackburn HL, Engler RJM, Vernalis MN. Cardiac lifestyle interventions differing in dietary stringency improve insulin resistance through changes in lipoprotein profiles. *J Am Coll Cardiol*. 2015;65(10_S). doi:10.1016/S0735-1097(15)61450-4.

American College of Cardiology (ACC) 64th Annual Scientific Session, San Diego, CA, March 2015. (Poster)

Abstract

Background: Metabolic dysfunction characterized by insulin resistance (IR) is an important risk factor for developing type-2 diabetes and coronary artery disease (CAD). The Lipoprotein Insulin Resistance (LP-IR) score, derived from measures of lipoprotein subclass particle concentration and size, is a new measure for assessing IR and identifying patients with increased risk for developing diabetes. Lifestyle modification interventions are known to mediate CAD risk through traditional measures such as blood pressure, lipids, and BMI; however, the effects of dietary stringency on IR and molecular drivers of the LP-IR score are unclear.
Methods: Patients with CAD or significant CAD risk factors participated in 1 of 2 clinical lifestyle interventions differing in dietary stringency: 1) an intensive non-randomized program with a strict vegetarian diet (n=90 subjects with 90 matched controls) and 2) a moderate randomized trial following a Mediterranean-style diet (n=90 participants, 58 controls). Changes over 1 year in lipoprotein profiles, LP-IR score, and traditional CAD risk factors were assessed by Wilcoxon Signed Rank tests.
Results: Participants in the intensive lifestyle intervention had poorer baseline cardiovascular health (significantly higher BMI, total cholesterol, triglycerides, LP-IR) than patients in the moderate program. Both interventions led to weight loss (-8.9%, intensive program; -2.6%, moderate program; $P<0.001$) and a significant decrease in LP-IR score (-13.3%, intensive; -7.9%, moderate; $P<0.01$) compared to respective controls over one year. Of the six lipoprotein parameters that comprise the LP-IR score, only large VLDL/chylomicrons decreased significantly in patients compared to controls in both programs (-26.3%, intensive; -13.7%, moderate; $P<0.05$).
Conclusions: Lifestyle modification including a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving insulin resistance defined by LP-IR. Significant reductions in large VLDL/chylomicrons may drive improvement in IR irrespective of dietary stringency.

Vernalis MN, Engler RJM, Mamula KA, Blackburn HL, Kashani M, Ellsworth DL. Weight loss impact on insulin resistance: A novel lipoprotein insulin resistance index (LP-IR) identifies differing phenotypes of response to lifestyle intervention. Military Health System Research Symposium (MHSRS), Fort Lauderdale, FL, August 2015. (Podium)

Abstract

Introduction: Lipoprotein Insulin Resistance Index (LP-IR) is a novel proprietary non-gender specific calculation for insulin resistance based on lipoprotein sub-particle size distribution. LP-IR is described as a reliable biomarker for progression to diabetes that reflects improvements in metabolic syndrome following dietary/lifestyle interventions with weight loss.

Objective: To compare post-diet/lifestyle intervention subjects who lost weight and decreased versus increased their LP-IR index.

Methods: Overweight/obese subjects with cardiovascular disease (CVD) or significant CVD risk factors enrolled in a 1 year intensive lifestyle intervention program including low fat (<10%) vegan diet. Risk factors, anthropometrics and biomarkers (including LP-IR, lipid profiles, etc.) associated with CVD risk were measured before and 1 year after intervention for comparison to weight loss changes. Subjects, stratified by LP-IR decrease or increase after 1 year, were compared using Wilcoxon nonparametric tests.

Results: Most participants (n=102, 49 males, 53 females) completed the program with weight-loss. Two groups were identified by LP-IR change: LP-IR score increase (25/102=24.5%); LP-IR decrease (77/102=75.5%). At baseline, there were no significant differences between these two LP-IR groups by age, BMI, systolic/diastolic BP, HDL/LDL/total cholesterol or triglycerides but mean LP-IR scores were significantly different (p=0.0019). Change in HDL-C, triglycerides, and LP-IR score after 1 year differed significantly between groups (p=0.0154, p=0.0024 and p=<0.0001, respectively).

	LP-IR Increased (N=25)			LP-IR Decreased (N=77)			Between Groups P-Value
Risk Factor	Baseline (SD)	Year 1 (SD)	% Change	Baseline (SD)	Year 1 (SD)	% Change	
BMI (kg/m^2)	33.556 (7.715)	30.02 (7.409)	-10.54%	33.82 (6.73)	30.561 (6.225)	-9.64%	0.4435
Systolic BP (mmHg)	134.16 (15.22)	126.56 (14.669)	-5.66%	137.143 (17.945)	128.182 (17.357)	-6.53%	0.7851
HDL-C (mg/dl)	48.32 (11.131)	43.96 (9.176)	-9.02%	44.532 (13.5)	43.688 (11.679)	-1.90%	0.0154
LDL-C (mg/dl)	120.375 (32.87)	108 (29.376)	-10.28%	109.613 (39.539)	106.133 (34.511)	-3.17%	0.0555
T-CHOL (mg/dl)	206.92 (39.841)	206.6 (116.635)	-6.34%	191.26 (46.04)	179.013 (41)	-6.40%	0.7088
TG (mg/dl)	183.08 (110.671)	206.6 (116.635)	12.85%	183.701 (91.098)	147.701 (78.629)	-19.60%	0.0024
LP-IR	58.24 (20.001)	66.72 (21.384)	14.56%	71.662 (16.126)	55.714 (18.773)	-22.25%	<.0001

Conclusion: The majority of individuals who lose weight reduce their LP-IR. However, a subgroup (25%) of patients increased their LP-IR despite weight loss. The clinical and prognostic significance of these observations require further study.

Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Vernalis MN. Cardiac interventions differing in lifestyle modification improve insulin resistance through changes in lipoprotein profiles. *Circulation* 2016;133:AP108.

AHA EPI/Lifestyle 2016 Scientific Sessions, Phoenix, AZ, March 2016. (Poster)

Abstract

Background: Metabolic dysfunction characterized by insulin resistance (IR) is an important risk factor for type-2 diabetes and coronary artery disease (CAD). The Lipoprotein Insulin Resistance (LP-IR) index, derived from measures of lipoprotein subclass particle concentration and size, is useful for assessing IR and identifying patients with increased diabetes/CAD risk.
Hypothesis: This study addressed the hypothesis that lifestyle modification programs differing in scope and intensity both improve IR through changes in lipoprotein profiles.
Methods: Patients with CAD or significant CAD risk factors participated in one of two clinical lifestyle interventions: 1) an intensive nonrandomized program with a strict vegetarian diet (n=90 subjects, 90 matched controls) or 2) a moderate randomized trial following a Mediterranean-style diet (n=89 participants, 58 controls). On-treatment and intention-to-treat analyses used regression modelling adjusted for CAD risk factors and lipid-lowering medication use to assess changes over one year in LP-IR, lipoprotein profiles, and CAD risk factors in intervention and control participants in both programs.
Results: Participants in the intensive lifestyle intervention had poorer baseline cardiovascular health than patients in the moderate program. In the on-treatment analysis, both lifestyle interventions led to weight loss [-8.9% (95% CI: -10.3, -7.4), intensive program; -2.8% (95% CI: -3.8, -1.9), moderate program; adjusted $p<0.001$] and a decrease in the LP-IR index [-13.3% (95% CI: -18.2, -8.3), intensive; -8.8% (95% CI: -12.9, -4.7), moderate; adjusted $p<0.01$] compared to respective controls over one year. Of the six lipoprotein parameters comprising LP-IR, only large very-low-density lipoprotein (VLDL) particle concentrations decreased significantly in patients compared to controls in both programs [-26.3% (95% CI: -43.0, -9.6), intensive; -14.2% (95% CI: -27.4, -1.0), moderate; $p<0.05$]. Intention-to-treat analysis confirmed and strengthened the primary results.

Discussion: In conclusion, moderate lifestyle modification following a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving IR defined by the LP-IR index. Significant reductions in large VLDL particles may drive improvement in IR irrespective of the magnitude of lifestyle changes.

Manuscripts:

Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Kashani M, Vernalis MN. Lifestyle modification interventions differing in intensity and dietary stringency improve insulin resistance through changes in lipoprotein profiles. *Diabetes, Obesity, and Metabolism. (in press)*

Manuscript Abstract

Aims: To determine if clinical lifestyle interventions differing in scope and intensity improve insulin resistance (IR), defined by the Lipoprotein Insulin Resistance (LP-IR) index, in patients differing in the severity of metabolic dysfunction.

Methods: Patients with diagnosed type-2 diabetes, coronary artery disease (CAD), or significant risk factors participated in one of two clinical lifestyle interventions: 1) intensive nonrandomized program with a strict vegetarian diet (N=90 subjects, 90 matched controls) or 2) moderate randomized trial following a Mediterranean-style diet (N=89 patients, 58 controls). On-treatment and intention-to-treat analyses assessed changes over one year in LP-IR, lipoprotein profiles, and metabolic risk factors in intervention patients and controls in both programs.
Results: In the on-treatment analysis, both interventions led to weight loss: [-8.9% (95% CI, -10.3 to -7.4), intensive program; -2.8% (95% CI, -3.8 to -1.9), moderate program; adjusted $p<0.001$] and a decrease in the LP-IR index [-13.3% (95% CI, -18.2 to -8.3), intensive; -8.8% (95% CI, -12.9 to -4.7), moderate; adjusted $p<0.01$] compared to respective controls. Of the six lipoprotein parameters comprising LP-IR, only large very-low-density lipoprotein (VLDL) particle concentrations decreased significantly in patients compared to controls in both programs [-26.3% (95% CI, -43.0 to -9.6), intensive; -14.2% (95% CI, -27.4 to -1.0), moderate; $p<0.05$]. Intention-to-treat analysis confirmed and strengthened the primary results.
Conclusions: Moderate lifestyle modification following a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving IR defined by the LP-IR index.

Important findings are presented below in Tables 3 and 4.

Table 3. Changes in LP-IR score, lipoprotein components of LP-IR, and cardiovascular risk factors during intensive lifestyle modification in participants and matched controls

Variable	Controls (n=90)			Participants (n=90)			
	Baseline	Year 1	% change	Baseline	Year 1	% change	Matched pairs p value[a]
LP-IR score	56.5 ± 19.5[b]	55.5 ± 20.6	-1.8	68.4 ± 18.7[b]	59.3 ± 20.2[d]	-13.3	<0.001
Lipoprotein components of LP-IR							
Lg VLDL/chylomicrons (nmol/L)	6.5 ± 7.0[c]	5.8 ± 5.8	-10.3	9.5 ± 7.5[c]	7.0 ± 7.6[d]	-26.3	0.034
Small LDL particles (nmol/L)	711 ± 306[c]	698 ± 324	-1.8	862 ± 342[c]	798 ± 328	-7.4	0.158
Lg HDL particles (μmol/L)	5.1 ± 2.8[b]	5.1 ± 3.0	-0.4	3.8 ± 2.4[b]	4.2 ± 2.4	+9.9	0.147
VLDL size (nm diameter)	51.1 ± 7.3[b]	50.3 ± 7.0	-1.6	55.1 ± 8.7[b]	50.9 ± 8.4[d]	-7.5	0.005
LDL size (nm diameter)	20.5 ± 0.6[c]	20.6 ± 0.6	+0.4	20.3 ± 0.7[c]	20.4 ± 0.6	+0.4	0.986
HDL size (nm diameter)	9.0 ± 0.4[c]	9.0 ± 0.4	-0.1	8.8 ± 0.4[c]	8.9 ± 0.4	+0.5	0.388
Dietary measures							
Calories (kcal)	1887 ± 580	1728 ± 495[e]	-8.4	2077 ± 768	1760 ± 479[e]	-15.3	0.153
Calories from fat (kcal)	606 ± 320	546 ± 225	-9.9	624 ± 376	239 ± 121[d]	-61.7	<0.001
Calories from saturated fat (kcal)	196 ± 113	174 ± 78[e]	-11.0	198 ± 137	50.3 ± 34.1[d]	-74.6	<0.001
Carbohydrates (g)	237 ± 72[c]	222 ± 76[e]	-6.3	281 ± 106[c]	317 ± 88[d]	+12.7	0.021

Dietary fiber (g)	18.5 ± 8.2[b]	17.2 ± 7.7	-7.0	26.2 ± 13.6[b]	39.9 ± 12.7[d]	+52.3	<0.001
Cardiovascular risk factors							
BMI (kg/m^2)	28.5 ± 4.5[b]	28.7 ± 4.8	+0.5	33.4 ± 7.4[b]	30.4 ± 7.0[d]	-8.9	<0.001
Diastolic BP (mm Hg)	79.4 ± 9.9	78.0 ± 8.8	-1.8	80.1 ± 10.0	75.4 ± 9.3[d]	-5.9	0.083
LDL (mg/dL)	108 ± 35	108 ± 35	0.0	109 ± 38	106 ± 33	-3.0	0.359
HDL (mg/dL)	49.8 ± 13.1[c]	48.1 ± 13.2[e]	-3.4	44.3 ± 12.7[c]	43.0 ± 10.7	-2.9	0.609
Total cholesterol (mg/dL)	188 ± 45	187 ± 43	-0.1	192 ± 47	182 ± 43[e]	-5.2	0.003
Triglycerides (mg/dL)	148 ± 98[c]	147 ± 82	-0.5	180 ± 92[c]	161 ± 89[e]	-11.0	0.019

Values are presented as mean ± SD.
[a] Based on a Wilcoxon Signed Rank test comparing change from baseline to year 1 in intensive lifestyle participants versus matched controls.
Baseline values in participants were significantly different from controls based on a Wilcoxon Signed Rank test for matched pairs: [b] P<0.001; [c] P<0.05.
Year 1 values were significantly different from baseline using a Wilcoxon Signed Rank test: [d] P<0.001; [e] P<0.05.

Table 4. Changes in LP-IR index, lipoprotein components of LP-IR, and cardiovascular risk factors during moderate lifestyle modification in participants and randomized controls

Variable	Controls (n=58)		Participants (n=90)		
	Baseline	Year 1 % change	Baseline	Year 1 % change	Between group p value[a]
LP-IR index	51.4 ± 23.4	+3.7	53.6 ± 20.7	-7.9[b]	0.002
Lipoprotein components of LP-IR					
Large VLDL particles (nmol/L)	4.1 ± 4.3	+3.8	4.7 ± 5.0	-13.7[c]	0.020
Small LDL particles (nmol/L)	774 ± 390	-5.6	769 ± 325	-12.7[b]	0.328
Large HDL particles (μmol/L)	5.4 ± 3.6	+0.1	5.2 ± 3.1	+10.1[c]	0.090
VLDL size (nm diameter)	48.3 ± 6.6	+2.3	49.1 ± 5.9	-0.4	0.125
LDL size (nm diameter)	20.6 ± 0.6	-0.1	20.5 ± 0.5	+0.3	0.350
HDL size (nm diameter)	9.0 ± 0.5	+0.1	9.0 ± 0.4	+1.0[c]	0.051
Dietary measures					
Calories (kcal)	1947 ± 644	-10.1[c]	1811 ± 602	-10.0[c]	0.405
Calories from fat (kcal)	640 ± 293	-9.3	573 ± 274	-13.2[c]	0.220
Calories from sat fat (kcal)	201 ± 99	-1.8	180 ± 84	-11.2[c]	0.097
Carbohydrates (g)	249 ± 81	-12.0[c]	233 ± 82	-8.4[c]	0.727

Dietary fiber (g)	21.1 ± 8.7	-2.6	22.1 ± 9.5	+0.9	0.287
Cardiovascular risk factors					
BMI (kg/m^2)	31.1 ± 6.5	-0.1	31.4 ± 6.5	-2.6[b]	<0.001
Diastolic BP (mm Hg)	78.9 ± 11.0	-1.6	79.9 ± 11.4	-6.2[b]	0.096
LDL (mg/dL)	116 ± 30	-4.8	111 ± 31	-3.3	0.319
HDL (mg/dL)	50.2 ± 14.5	-2.3[c]	47.7 ± 12.3	+0.5	0.063
Total cholesterol (mg/dL)	192 ± 36	-4.3[c]	185 ± 39	-3.2	0.493
Triglycerides (mg/dL)	130 ± 64	-3.1	135 ± 66	-10.4[b]	0.054

Values are presented as mean ± SD.
[a] Based on a Wilcoxon nonparametric test comparing change from baseline to year 1 in moderate lifestyle participants versus randomized controls.
Year 1 values were significantly different from baseline using a Wilcoxon Signed Rank test: [b] P<0.001; [c] P<0.05.

Macrophage migration inhibitory factor (MIF) – MIF is an inflammatory cytokine that regulates smooth muscle cell migration and proliferation, and thus plays an important role in promoting development of atherosclerotic lesions. MIF has been shown to be an important biomarker for diseases with inflammation, such as CVD, diabetes, obesity, and cancer. A draft manuscript summarizing results has been prepared and additional revisions are needed.

Genotyping of genetic variants in MIF gene that influence circulating levels is complete; data analysis complete. MIF levels decreased significantly ($p<0.05$) in Ornish participants compared to controls at 12 weeks, but no difference in MIF levels between cases and controls was seen at one year. Only women participants showed significant ($p<0.05$) reductions in MIF levels at 12 weeks (-23%). No change in men. Transcription of the human MIF gene is regulated by genetic polymorphisms in the MIF promoter, including the –173G/C single-nucleotide polymorphism and a sequence of tetra-nucleotide repeats at –794 (-794CATT$_{5-8}$). These polymorphisms may have relevance to cardiovascular disease, and this area has become a growing area of investigation; however, the tetranucleotide polymorphism and SNP variants were too infrequent for meaningful analysis.

Abstract Presented:

Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. *Circulation* 2012;126:A14216.

AHA Scientific Sessions 2012, Los Angeles, CA, November 2012. (Poster)

Abstract

Background: Inflammation and gender are key factors in cardiovascular disease (CVD) pathogenesis and outcomes. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that contributes to CVD risk through inflammatory vulnerable plaque

formation, while CRP is a systemic marker of inflammation. Lifestyle modification programs focusing on nutrition, exercise, and stress management are effective in mediating CVD risk through traditional measures like weight, blood pressure, and lipids; however, little is known about gender-related differences and response of emerging risk factors such as MIF to lifestyle modification.

Methods: In a prospective study of patients with elevated CVD risk matched to controls by age, gender and CVD risk factors (n=85/group), we investigated 1) changes in circulating MIF and HS-CRP, 2) the influence of gender on changes in MIF and HS-CRP, 3) correlation between changes in MIF and HS-CRP during an intensive CVD risk reduction program.

Results: Baseline MIF and HS-CRP were higher in women vs. men (P=0.04) in patients enrolled in the lifestyle modification program (MIF: 3.1 ± 1.9 vs. 2.8 ± 1.9 ng/ml; HS-CRP: 5.9 ± 7.7 vs. 3.5 ± 2.7 ng/ml) and controls (MIF: 3.3 ± 2.0 vs. 2.5 ± 1.7 ng/ml; HS-CRP: 3.7 ± 3.6 vs. 1.6 ± 2.0 ng/ml). After 3 months of lifestyle modification, female gender accounted for the majority of decrease in MIF and HS-CRP. Women showed a 23% decrease in MIF (3.1 ± 1.9 vs. 2.4 ± 1.2 ng/ml, P=0.05) and a 40% decrease in HS-CRP (5.9 ± 7.7 vs. 3.5 ± 4.5 ng/ml, P=0.06), but neither MIF nor HS-CRP changed significantly in controls or men in the lifestyle modification program. Pair wise correlation did not show a relationship between changes in MIF and HS-CRP.

Conclusions: Pro-inflammatory MIF and HS-CRP decreased in response to intensive diet/lifestyle intervention, with improvement being more evident in women than men. While changes in weight and blood pressure were similar in both genders during the lifestyle intervention, changes in inflammatory markers were dependent on gender. This suggests intensive lifestyle modification may lessen CVD risk in women through different mechanisms than in men.

Gene Expression – A manuscript comparing changes in gene expression in Ornish versus control participants was published. The reference and key findings of the study are provided below.

Manuscript:

Ellsworth DL, Croft DT Jr, Weyandt J, Sturtz LA, Blackburn HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in expression of genes and pathways important to vascular function. *Circ Cardiovasc Genet* 2014;7:151-160.

Manuscript Abstract

Background—Healthy lifestyle changes are believed to mediate cardiovascular disease (CVD) risk through pathways affecting endothelial function and progression of atherosclerosis; however, the extent, persistence, and clinical significance of molecular change during lifestyle modification are not well known. We examined the impact of a rigorous CVD risk reduction program on peripheral blood gene expression profiles in 63 participants and 63 matched controls to characterize molecular responses and identify regulatory pathways important to cardiovascular health.

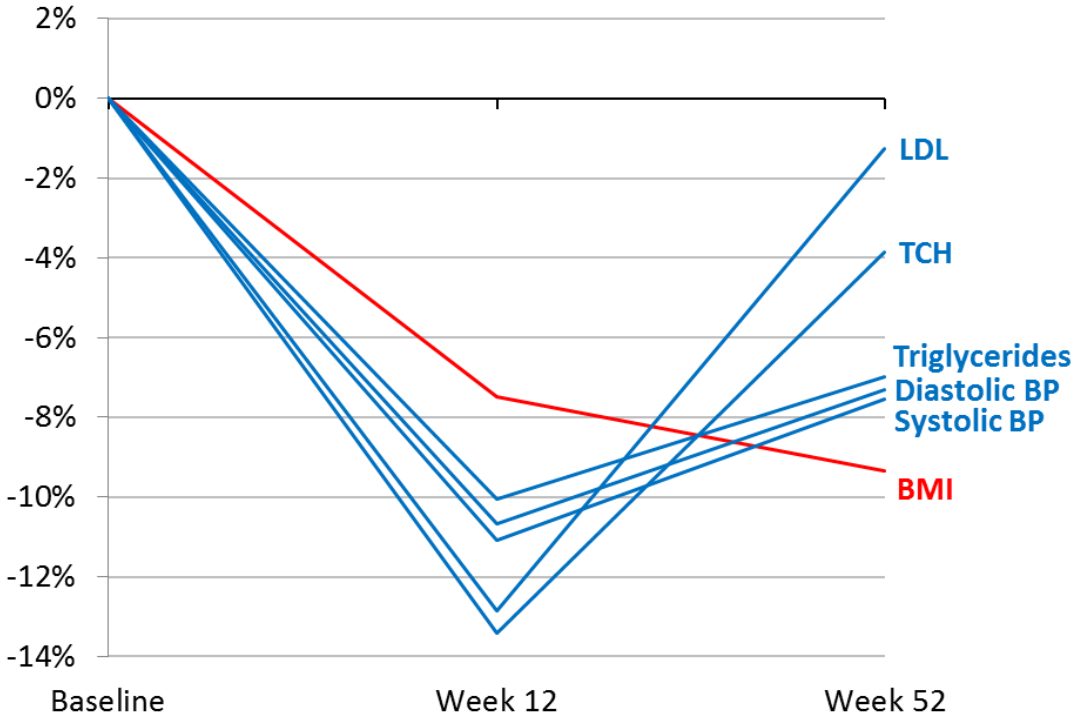
Methods and Results—Dramatic changes in dietary fat intake (-61%, $P<0.001$ versus controls) and physical fitness (+34%, $P<0.001$) led to significant improvements in CVD risk factors. ANOVA with FDR-correction for multiple testing ($P<0.05$) identified 26 genes after 12 weeks

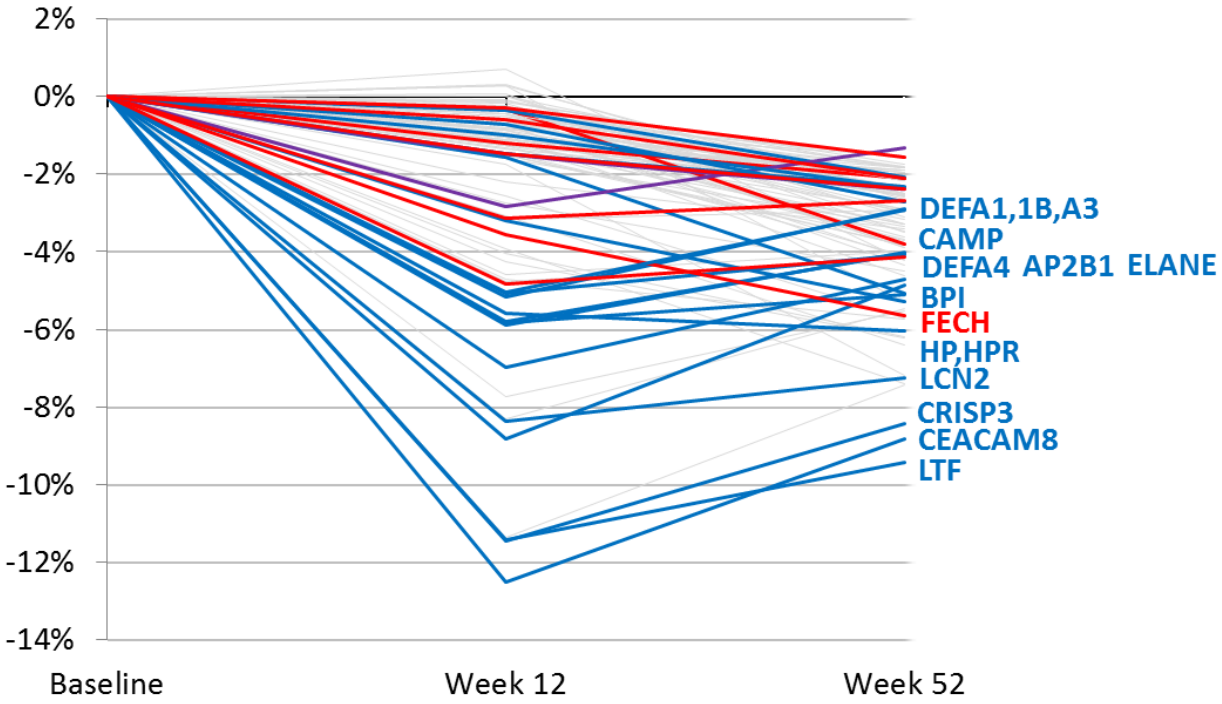
and 143 genes after 52 weeks that were differentially-expressed from baseline in participants. Controls showed little change in CVD risk factors or gene expression. Quantitative RT-PCR validated differential expression for selected transcripts. Lifestyle modification effectively reduced expression of proinflammatory genes associated with neutrophil activation and molecular pathways important to vascular function, including cytokine production, carbohydrate metabolism, and steroid hormones. Prescription medications did not significantly affect changes in gene expression.
Conclusions—Successful and sustained modulation of gene expression through lifestyle changes may have beneficial effects on the vascular system not apparent from traditional risk factors. Healthy lifestyles may restore homeostasis to the leukocyte transcriptome by down-regulating lactoferrin and other genes important in the pathogenesis of atherosclerosis.
Clinical Trial Registration—URL: www.clinicaltrials.gov. Unique identifier: NCT01805492

The manuscript showed that changes in gene expression mirrored changes in many CVD risk factors (Fig. 3 below) – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52 (Fig. 4 below). Most cholesterol and lipid homeostasis genes showed a continual decrease in expression throughout the program similar to body weight (Fig. 4 below). Medication use clearly did not affect gene expression, thus expression changes may be attributed to the lifestyle change program (Table 5 below).

Throughout the program, many genes exhibiting the largest fold changes in expression were significantly correlated with BMI (Fig. 5 below). Notably, few genes correlated with blood pressure or plasma lipids after 12 weeks.

To better understand vascular responses to lifestyle modification, we compared genes that were differentially regulated during CVD risk reduction to expression signatures reported for major leukocyte subpopulations. Genes influenced by lifestyle change were expressed in several cell populations, suggesting that different types of circulating cells with unique and specialized functions may be involved in vascular responses to lifestyle modification (Fig. 6 below).





Figures 3 and 4. Change in traditional CVD risk factors (Fig. 3) and changes in gene expression (Fig. 4) during intensive lifestyle change.

Table 5. Effects of medications on gene expression from Baseline to Week 52

Probe ID	Symbol	Fold Change All Participants (n=63)	Fold Change Lipid Lowering Medications* (n=51)	Fold Change All Medications† (n=34)
202018_s_at	LTF	–1.67	–1.67	–1.70
221748_s_at	TNS1	–1.55	–1.51	–1.43
212531_at	LCN2	–1.47	–1.44	–1.48
206676_at	CEACAM8	–1.44	–1.48	–1.68
214407_x_at	GYPB	–1.41	–1.34	–1.26
206698_at	XK	–1.41	–1.43	–1.36
206665_s_at	BCL2L1	–1.39	–1.35	–1.31
203502_at	BPGM	–1.37	–1.40	–1.41
203115_at	FECH	–1.35	–1.31	–1.28
207802_at	CRISP3	–1.32	–1.32	–1.43
208470_s_at	HP/HPR	–1.30	–1.31	–1.24
212768_s_at	OLFM4	–1.29	–1.20	–1.23
213446_s_at	IQGAP1	–1.28	–1.25	–1.22
208632_at	RNF10	–1.28	–1.25	–1.18
221627_at	TRIM10	–1.28	–1.23	–1.21
218418_s_at	KANK2	–1.28	–1.22	–1.21
217878_s_at	CDC27	–1.27	–1.26	–1.22
210244_at	CAMP	–1.27	–1.26	–1.27
200615_s_at	AP2B1	–1.26	–1.24	–1.22
205557_at	BPI	–1.25	–1.22	–1.29
211993_at	WNK1	–1.25	–1.23	–1.17

B-W12	BMI −7.5%	SBP −11.1%	DBP −10.7%	LDL −12.9%	TCH −13.4%	TG −10.1%	EC +28.1%
LTF	+0.44	+0.12	+0.18	+0.23	+0.23	+0.11	−0.09
TNS1	+0.11	+0.01	+0.05	−0.06	−0.13	−0.17	+0.09
LCN2	+0.32	+0.12	+0.08	+0.09	+0.09	+0.08	−0.01
CEACAM8	+0.47	+0.06	+0.03	+0.32	+0.26	+0.09	−0.03
GYPB	+0.04	−0.04	−0.05	−0.05	−0.10	−0.11	+0.13
XK	+0.04	−0.02	−0.02	−0.03	−0.09	−0.18	+0.17
BCL2L1	−0.04	+0.03	+0.07	−0.06	−0.15	−0.27	+0.10
BPGM	+0.10	−0.06	+0.01	−0.01	−0.05	−0.11	+0.18
FECH	+0.09	−0.04	+0.01	−0.12	−0.15	−0.12	+0.12
CRISP3	+0.41	+0.02	+0.06	+0.21	+0.28	+0.27	−0.14
HP/HPR	+0.32	+0.07	+0.24	+0.22	+0.24	+0.01	−0.01
OLFM4	+0.43	−0.01	+0.05	+0.29	+0.29	+0.20	+0.06
IQGAP1	+0.03	+0.02	+0.20	+0.04	−0.02	−0.27	−0.11
RNF10	+0.07	−0.03	+0.04	−0.09	−0.19	−0.26	+0.05
TRIM10	+0.05	−0.06	−0.05	−0.06	−0.16	−0.25	+0.10
KANK2	−0.01	−0.07	−0.01	−0.12	−0.20	−0.19	+0.09
CDC27	+0.05	−0.10	+0.01	−0.10	−0.15	−0.26	−0.07
CAMP	+0.29	0.00	+0.04	+0.13	+0.18	+0.17	−0.01
AP2B1	+0.07	−0.09	+0.15	+0.02	−0.09	−0.27	+0.07
BPI	+0.34	+0.21	+0.17	+0.10	+0.07	−0.02	+0.03
WNK1	+0.01	−0.03	+0.03	−0.12	−0.19	−0.25	+0.09

W12-52	BMI −2.0%	SBP +4.0%	DBP +3.8%	LDL +13.3%	TCH +11.1%	TG +3.4%	EC +8.0%
LTF	+0.24	+0.12	+0.06	−0.19	−0.11	+0.23	−0.26
TNS1	+0.39	+0.11	+0.03	−0.14	−0.09	+0.11	−0.32
LCN2	+0.29	+0.12	+0.05	−0.23	−0.19	+0.07	−0.34
CEACAM8	+0.27	+0.06	+0.01	−0.18	−0.14	+0.15	−0.26
GYPB	+0.50	+0.07	+0.03	−0.24	−0.22	0.00	−0.39
XK	+0.46	+0.06	−0.01	−0.22	−0.22	−0.02	−0.36
BCL2L1	+0.33	+0.05	−0.03	−0.07	−0.06	+0.02	−0.31
BPGM	+0.49	+0.03	−0.05	−0.20	−0.18	−0.01	−0.35
FECH	+0.48	+0.09	−0.01	−0.18	−0.18	−0.01	−0.37
CRISP3	+0.26	−0.08	−0.05	−0.22	−0.20	+0.08	−0.18
HP/HPR	+0.32	−0.05	−0.03	−0.32	−0.27	+0.09	−0.18
OLFM4	+0.16	−0.01	0.00	−0.15	−0.13	+0.14	−0.17
IQGAP1	0.00	+0.26	+0.18	+0.10	+0.11	+0.10	−0.03
RNF10	+0.29	+0.12	+0.04	−0.16	−0.12	+0.09	−0.33
TRIM10	+0.36	+0.07	−0.02	−0.21	−0.20	+0.02	−0.38
KANK2	+0.37	+0.08	−0.09	−0.12	−0.12	−0.02	−0.35
CDC27	+0.13	+0.12	+0.04	−0.03	−0.01	+0.05	−0.32
CAMP	+0.34	+0.02	+0.03	−0.31	−0.29	−0.02	−0.29
AP2B1	+0.26	+0.08	−0.05	−0.08	−0.04	+0.15	−0.29
BPI	+0.25	+0.17	+0.08	−0.29	−0.27	+0.02	−0.24
WNK1	+0.14	+0.16	0.00	+0.03	+0.07	+0.18	−0.17

B-W52	BMI −9.4%	SBP −7.6%	DBP −7.3%	LDL −1.3%	TCH −3.9%	TG −7.0%	EC +38.4%
LTF	+0.46	+0.16	+0.08	+0.18	+0.25	+0.14	−0.35
TNS1	+0.38	+0.13	+0.01	+0.05	+0.08	+0.01	−0.26
LCN2	+0.34	+0.20	+0.05	+0.15	+0.16	+0.08	−0.30
CEACAM8	+0.38	+0.10	+0.07	+0.32	+0.33	+0.06	−0.30
GYPB	+0.39	+0.14	+0.05	0.00	−0.02	−0.04	−0.25
XK	+0.32	+0.12	+0.09	+0.07	+0.05	−0.11	−0.20
BCL2L1	+0.18	+0.10	−0.12	+0.08	+0.06	−0.06	−0.21
BPGM	+0.35	+0.11	+0.10	+0.09	+0.07	−0.13	−0.21
FECH	+0.32	+0.16	+0.01	+0.08	+0.07	−0.09	−0.18
CRISP3	+0.33	−0.02	+0.12	+0.20	+0.24	+0.12	−0.18
HP/HPR	+0.46	+0.01	+0.25	+0.14	+0.04	−0.11	−0.10
OLFM4	+0.35	−0.02	+0.06	+0.15	+0.14	+0.02	−0.18
IQGAP1	+0.20	+0.12	+0.04	+0.02	+0.01	0.00	−0.33
RNF10	+0.38	+0.10	−0.05	+0.02	+0.05	+0.03	−0.29
TRIM10	+0.35	+0.12	0.00	0.00	0.00	−0.10	−0.22
KANK2	+0.32	+0.09	−0.10	+0.02	+0.02	−0.12	−0.21
CDC27	+0.11	−0.02	−0.11	−0.16	−0.16	−0.01	−0.28
CAMP	+0.37	+0.15	+0.21	+0.17	+0.16	+0.04	−0.10
AP2B1	+0.41	+0.07	−0.13	+0.04	+0.05	−0.05	−0.26
BPI	+0.29	+0.23	+0.17	+0.24	+0.19	−0.07	−0.18
WNK1	+0.20	+0.08	−0.06	−0.06	−0.03	−0.02	−0.16

Figure 5. Pair-wise correlations for changes in CVD risk factors and gene expression from baseline to 12 weeks (top), week 12 to week 52 (middle), and baseline to week 52 (bottom) during intensive lifestyle modification. Coefficients highlighted in dark green were significant at $P<0.001$, light green $P<0.05$. Risk factor percent changes are the group averages from Table 1. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity. Stringent gene list of changes at 52 weeks with combined significance (FDR $P<0.05$) and expression change ($\geq$1.25-fold) filtering.


Gene
CD8+ T-Cells *
Lymphocytes +
Lymphocytes *
Neutrophils +
B-Cells *
CD4+ T-Cells *
T-Cells ++
CD8+ T-Cells §
Reticulocytes +
Monocytes ++
CD4+ T-Cells §
Granulocytes *
CD3+ T-Cells §
Acute Phase Proteins
Trauma/Burn ++
Systemic Inflammation
Severe Trauma #
LTF
TNS1
LCN2
CEACAM8
GYPB
XK
BCL2L1
BPGM
FECH
CRISP3
HP/HPR
OLFM4
IQGAP1
KANK2
RNF10
TRIM10
CAMP
CDC27
AP2B1
BPI
GYPB/GYPE
WNK1
HP
SPTB
TCN1
CLPTM1
DSC2
MMP8
RETN
CEACAM6
MICAL2
MKRN1
SIAH2
CLEC5A
PPP3R1
PSME4
S100A12
ADRBK2
KLHL24
TRAK2
AGFG1
C1orf144
CD36
SP100
ALDH5A1
CTSB
CYP1B1
EZR
GAPVD1
MAP4K5
MTMR3
PAK2
SORT1
CREG1
CTNNA1
DENND1A
FRMD4A
MAPK1
MEGF9
PDLIM5
SCD
STXBP2
ALDH2
CYBB
CYFIP1
DPYSL2
EMR1
HK1
LDLR
MKLN1
PLBD1
Gene
CD8+ T-Cells *
Lymphocytes +
Lymphocytes *
Neutrophils +
B-Cells *
CD4+ T-Cells *
T-Cells ++
CD8+ T-Cells §
Reticulocytes +
Monocytes ++
CD4+ T-Cells §
Granulocytes *
CD3+ T-Cells §
Acute Phase Proteins
Trauma/Burn ++
Systemic Inflammation
Severe Trauma #
TNS3
TPR
ASGR2
CBARA1
CSNK2A1
IRAK3
KIAA0494
PSMD1
TMEM176B
TPM4
C3AR1
C6orf106
FBN2
GAS7
GNA15
KCTD12
LSM12
PLCL2
RALBP1
RRAGD
SLAMF7
SNX3
SRD5A1
TJP2
TMPO
TSPAN4
ATP2A2
DDX6
NBN
TMEM176A
OTUD3
PFN2
TUBG2
C16orf67
EVL
FGF9
FNBP4
IL11RA
LUC7L3
MLL
PHF1
RAB40B
RPL10
RPL37A
TNFRSF25
ZNF248
ANK3
ENO2
FAM153A/B/C
HNRNPL
HOXB2
LOC730092
LPIN1
ME3
NACA
REV1
SFRS17A
SFRS7
TAF1D
ENOSF1
HNRNPH3
LOC150759
PURA
GPRASP1
LOC729799
SIP1
ZDHHC11
RRN3
PTCH1
ZFAND6
ZNF204

Figure 6. Congruence between CVD risk reduction genes and expression signatures reported for major leukocyte subpopulations or CVD-relevant processes. Squares denote whether genes differentially regulated after 52 weeks of intensive lifestyle modification also were expressed (green squares) or not expressed (red squares) in published profiles.

Weight loss. Additional analyses of the gene expression data were conducted by stratifying patients by weight loss. Patients experiencing substantial weight loss lost an average of 15.2 $\pm$ 3.8% of their total body weight from baseline to 1 year, while those attaining only minimal weight loss lost an average of 3.1 $\pm$ 2.5% of body weight. Participants in both the substantial and minimal weight loss groups experienced a significant increase in exercise capacity and carbohydrate intake, and a significant decrease in dietary fat ($P<0.001$) compared to matched controls. Patients losing substantial weight also showed significant reductions in blood pressure, triglycerides, and total caloric intake versus controls. Compared to the minimal weight loss group, participants losing substantial weight significantly increased their exercise capacity and dietary carbohydrates, but decreased total calories and fat intake during the program.

Abstract Presented:

Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. The importance of weight loss for effecting molecular change during intensive cardiovascular risk reduction. Obesity 2012: 30th Annual Scientific Meeting, San Antonio, TX, September 2012. (Poster)

Abstract

Obesity is a major risk factor for cardiovascular (CV) disease. Behavioral lifestyle change is the cornerstone of therapy for weight management. Currently little is known about molecular responses accompanying weight loss that may be important in weight control and CV risk reduction. Patients (n=89) participated in a prospective, nonrandomized, lifestyle change program designed to stabilize or reverse progression of CV disease through dietary changes, exercise, and stress reduction. Nonintervention controls (n=63) were matched to patients based on age, gender, and disease status. CV risk factors (BMI, blood pressure, lipids) and peripheral blood gene expression profiles were assessed at three time points over one year. Most patients were obese (63%; BMI>30) or overweight (25%; 25<BMI<30 kg/m2) at baseline, but showed significant improvement in CV risk factors compared to controls during the program. Following stratification based on weight loss, we observed significant expression changes (FDR P<0.05) for 41 genes in participants who lost the most weight (mean weight loss=11%) from baseline to three months and for 3223 genes in those who lost the most weight (mean weight loss=15%) from baseline to one year. No significant expression changes were observed in patients who lost the least weight (mean weight loss<4%) or in controls. Functional ontologies of genes showing the most significant changes in expression included immune/defense response and symbiosis at three months and metabolism/biosynthesis at one year. Intensive lifestyle modification can effectively alter CV risk factors, but successful weight loss may accentuate molecular change. Defining the role of weight loss in molecular response to lifestyle modification provides another dimension to understanding complex biological processes involved in CV health.

Additional analyses of the gene expression data have been conducted with patients stratified by weight loss. A manuscript describing these results was published in the journal *Obesity*. The citation, abstract, and important findings from the paper are provided below:

Manuscript:

Ellsworth DL, Mamula KA, Blackburn HL, McDyer FA, Jellema GL, van Laar R, Costantino NS, Engler RJ, Vernalis MN. Importance of substantial weight loss for altering gene expression during intensive cardiovascular lifestyle modification. *Obesity (Silver Spring)* 2015 Jun;23(6):1312-9. doi: 10.1002/oby.21079. Epub 2015 May 9.

Manuscript Abstract

Objective: To examine relationships between weight loss through changes in lifestyle and peripheral blood gene expression profiles.

Methods: A prospective nonrandomized trail was conducted over 1 year in participants undergoing intensive lifestyle modification to reverse or stabilize progression of coronary artery disease. Cardiovascular risk factors, inflammatory biomarkers, and gene expression as a function of weight loss were assessed in 89 lifestyle participants and 71 retrospectively matched controls undergoing usual care.

Results: Substantial weight loss (-15.2+3.8%) in lifestyle participants (n=33) was associated with improvement in selected cardiovascular risk factors and significant changes in peripheral blood gene expression from pre- to post-intervention: 132 unique genes showed significant expression changes (false discovery rate corrected P-value <0.05 and fold-change >1.4). Altered molecular pathways were related to immune function and inflammatory responses involving endothelial activation. In contrast, participants losing minimal weight (-3.1+2.5%, n=32) showed only minor changes in cardiovascular risk factors and markers of inflammation, and no changes in gene expression compared to non-intervention controls after 1 year.

Conclusions: Weight loss (>10%) during lifestyle modification is associated with down-regulation of genetic pathways governing interactions between circulating immune cells and the vascular endothelium and may be required to successfully reduce CVD risk.

The average age of intervention participants (45 women and 44 men) was 60.4 years (range 40.7-85.0) and the average age of controls (36 women and 35 men) was 60.6 years (range 40.6-79.7). Despite the prospective matching strategy, participants and controls differed for some variables at baseline: lifestyle participants were heavier ($P < 0.001$), consumed a higher percentage of carbohydrates ($P = 0.034$), had lower exercise capacity ($P < 0.001$), and higher triglyceride ($P = 0.004$) and leptin ($P = 0.019$) levels.

Patients experiencing substantial weight loss lost an average of 15.2+3.8% of their total body weight from baseline to 1 year, while those attaining only minimal weight loss lost an average of 3.1+2.5% of body weight (Table 6 below). Patients losing substantial weight also showed significant improvement in dietary measures, diastolic blood pressure, exercise capacity, triglycerides, insulin, and leptin versus controls. Participants in the minimal weight loss group showed significant changes only for carbohydrate and fat consumption and exercise capacity, but experienced no significant changes in blood pressure, plasma lipids, or inflammatory markers compared to controls (Table 6 below).

At baseline, no genes showed a significant difference in expression between participants and matched controls using an FDR-corrected P–value of <0.05. Using the MD Anderson Cancer Center sample size calculator (http://bioinformatics.mdanderson.org/MicroarraySampleSize/), with 33 patients in the substantial weight loss group, we had 80% power to detect a >1.4 fold-change in gene expression. During 1 year of intensive lifestyle modification, molecular change occurred with successful weight loss — 132 unique genes changed significantly in expression (FDR-corrected P < 0.05, fold-change >1.4). No expression changes were observed in participants who lost minimal weight or in nonintervention controls. Validation experiments showed a strong positive correlation (r = 0.964, P < 0.0001) across all 8 genes between fold-changes determined by qRT-PCR and microarray analysis.

In addition to individual genes, Gene Set Enrichment Analysis detected 7 molecular pathways that were significantly down-regulated during successful weight loss. Many of these pathways influence interactions between circulating leukocytes and the vascular endothelium, cellular adhesion, and neutrophil granulation, which are processes important in vascular inflammation.

Table 6 Change in dietary measures, CVD risk factors, and plasma biomarkers over 1 year in lifestyle participants and matched controls stratified by weight loss success

		Controls		Participants		
Measure	Weight Loss Group[a]	Baseline	One Year % Change	Baseline	One Year % Change	Participants vs Controls *P*-value[b]
Weight (kg)	High	78.8 ± 14.3[d]	0.0	100.2 ± 19.6[d,e]	-15.2[*]	<0.001
	Low	87.9 ± 15.1	+1.1	91.9 ± 24.3[e]	-3.1[*]	<0.001
BMI (kg/m^2)	High	27.6 ± 3.7[d]	+0.3	34.7 ± 6.6[d]	-15.2[*]	<0.001
	Low	29.9 ± 3.9	+1.3	32.3 ± 8.7	-3.0[*]	<0.001
Dietary Measures						
Calories (kcal)	High	1635 ± 548[d]	-2.6	2188 ± 850[d]	-25.3[†]	0.008
	Low	1937 ± 659	-14.3[†]	1937 ± 738	-5.7	0.324
% Carbs[c]	High	50.1 ± 9.0	-3.3	52.7 ± 10.9	+36.8[*]	<0.001
	Low	47.7 ± 10.8[d]	+5.2[†]	56.9 ± 11.6[d]	+24.0[*]	<0.001
% Fat[c]	High	31.1 ± 9.6	+5.8	30.8 ± 10.3[e]	-63.1[*]	<0.001
	Low	34.1 ± 8.8[d]	-5.8	25.8 ± 9.8[d,e]	-54.7[*]	<0.001
Traditional Risk Factors						
SBP (mm Hg)	High	134 ± 18	-4.3[†]	135 ± 16	-7.3[†]	0.351
	Low	138 ± 19	-8.3[*]	139 ± 17	-7.4[†]	0.775

DBP (mm Hg)	High	77.9 ± 9.2	-1.1	81.2 ± 11.1	-10.2*	0.008
	Low	81.7 ± 8.6	-5.1†	81.8 ± 9.5	-7.2†	0.374
EC (Bruce)	High	9.9 ± 3.0[d]	+0.7	6.8 ± 2.0[d]	+44.0*	<0.001
	Low	9.7 ± 2.8[d]	-0.9	6.8 ± 2.4[d]	+28.3*	<0.001
LDL (mg/dl)	High	109 ± 38	-2.1	112 ± 40	-0.2	0.792
	Low	115 ± 37	-1.0	112 ± 40	-1.2	0.972
TCH (mg/dl)	High	191 ± 52	-0.7	193 ± 43	-4.3	0.447
	Low	193 ± 42	+0.4	191 ± 50	-2.6	0.469
TG (mg/dl)	High	144 ± 108[d]	+9.8	190 ± 107[d]	-16.9†	0.022
	Low	135 ± 52[d]	+11.7	166 ± 65[d]	+3.2	0.602
Plasma Biomarkers						
CRP (□g/ml)	High	2.2 ± 1.5[d]	-2.0	4.1 ± 3.5[d]	-32.1*	0.071
	Low	3.6 ± 5.1	-15.1	4.8 ± 7.3	-31.5	0.269
Insulin (□l	High	13.8 ± 6.3[d]	+3.4	21.5 ± 12.4[d,e]	-35.1*	<0.001
	Low	16.9 ± 7.9	+4.7	15.0 ± 9.3[e]	-1.5	0.540
Leptin (ng/ml)	High	16.9 ± 12.1[d]	+5.4	24.5 ± 15.2[d]	-50.9*	<0.001
	Low	20.3 ± 20.9	+12.1	24.0 ± 24.8	-10.4	0.101

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; CRP, C-reactive protein. Data are mean ± SD.

[a] High, substantial weight loss (n=33); Low, minimal weight loss (n=32).

[b] Based on a matched pairs t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers) comparing changes from baseline to 1 year in participants vs matched controls.

[c] % Carbs is percentage of total calories from carbohydrates; % Fat is percentage of total calories from fat.

[d] Participants and controls significantly different at baseline ($P<0.05$) based on a Wilcoxon Signed Rank test for matched pairs.

[e] Substantial weight loss and minimal weight loss participants significantly different at baseline ($P<0.05$) based on a Wilcoxon nonparametric test.

* $P<0.001$ compared to baseline using a paired t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers).

† $P<0.05$ compared to baseline using a paired t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers).

We summarized all of our gene expression research in a paper published in *Genomics Data*. The objective of this paper was to describe our data generation strategies and methods, data QC metrics, data analysis process and algorithms, biological interpretation and conclusions. This article describes our publicly available genomic datasets so that the data can be reproduced, reused, and reanalyzed.

Manuscript:

Blackburn HL, McErlean S, Jellema GL, van Laar R, Vernalis MN, Ellsworth DL. Gene expression profiling during intensive cardiovascular lifestyle modification: Relationships with vascular function and weight loss. *Genomics Data* 2015;4:50-53.
http://dx.doi.org/10.1016/j.gdata.2015.03.001

Manuscript Abstract

Heart disease and related sequelae are a leading cause of death and healthcare expenditure throughout the world. Although many patients opt for surgical interventions, lifestyle modification programs focusing on nutrition and exercise have shown substantial health benefits and are becoming increasing popular. We conducted a year-long lifestyle modification program to mediate cardiovascular risk through traditional risk factors and to investigate how molecular changes, if present, may contribute to long-term risk reduction. Here we describe the lifestyle intervention, including clinical and molecular data collected, and provide details of the experimental methods and quality control parameters for the gene expression data generated from participants and non-intervention controls. Our findings suggest successful and sustained modulation of gene expression through healthy lifestyle changes may have beneficial effects on vascular health that cannot be discerned from traditional risk factor profiles. The data are deposited in the Gene Expression Omnibus, series GSE46097 and GSE66175.

The figures below indicate that there was limited variability attributable to laboratory procedures across all arrays (Fig. 7) and that comparable percent present values (median=59.2%, range 48.1-64.8%), assessed using the mean absolute deviation, were observed for all samples (Fig. 8).

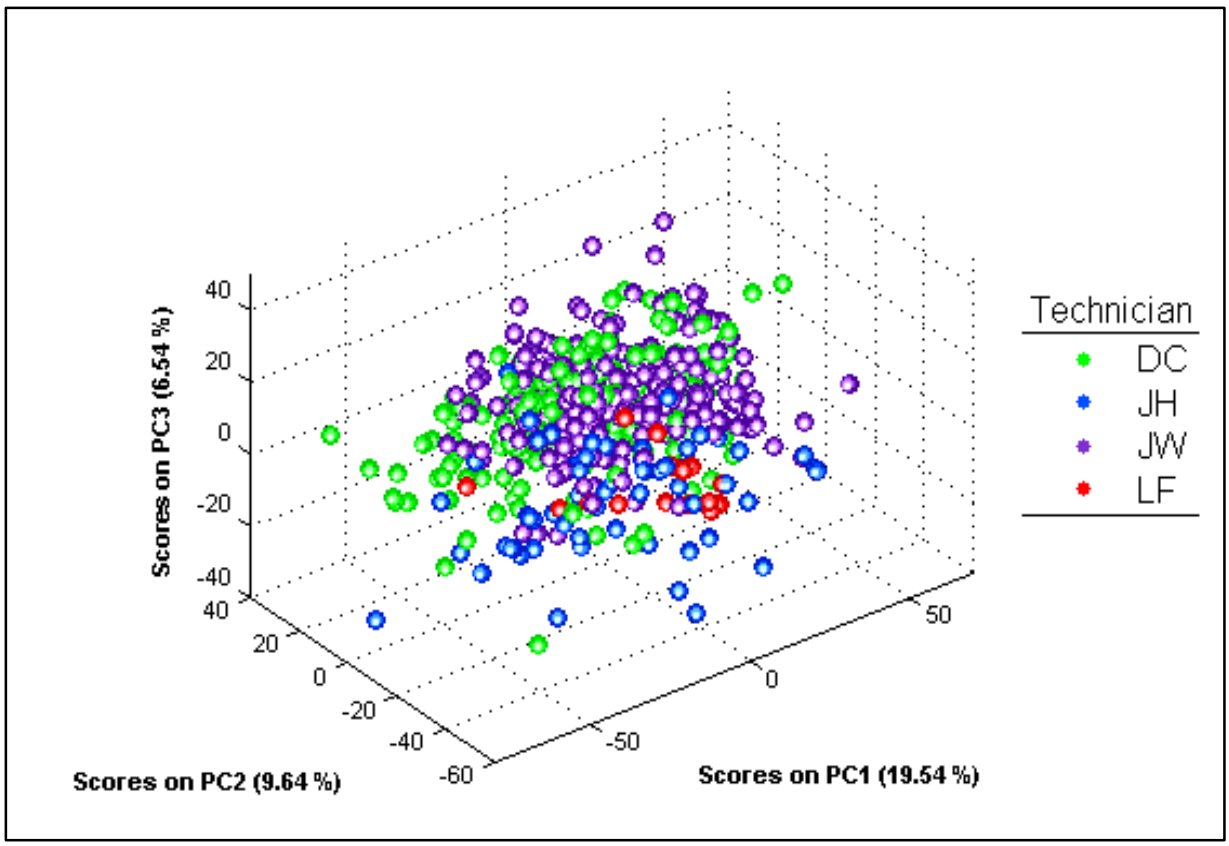


Figure 7. Three-dimensional scatter plot representing a Principal Component Analysis of all expression arrays colored by laboratory technician.

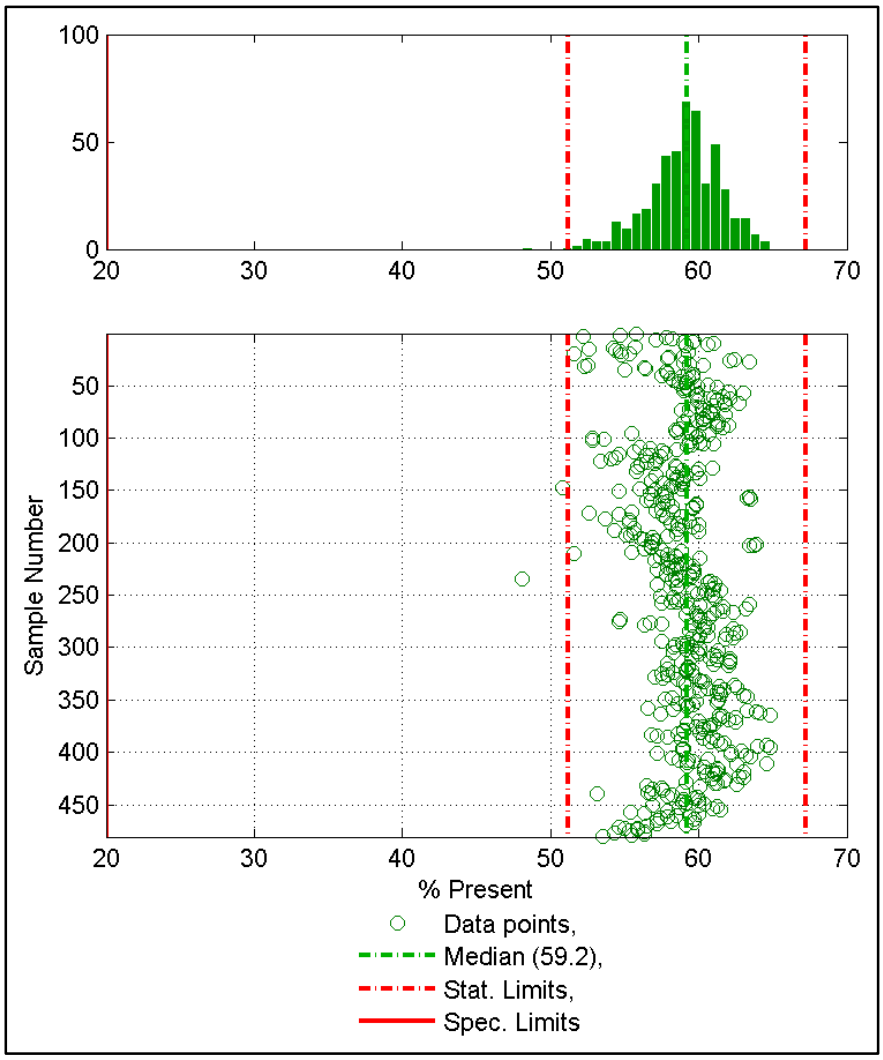


Figure 8. Histogram (top panel) and scatter plot (bottom panel) showing the percentage of probes on each array yielding detectable expression (percent present calls). The median percent present calls is represented by the dashed green line and statistical limits [±3.5 x STD (mean absolute deviation)] by the dashed red lines.

Structural and Functional Measures of Cardiovascular Health – Specific endpoints measured include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. PET/CT scanning analysis continues; collaboration ongoing with Dr. Edward Miller, Boston University to provide clinical insight into data. Results of the initial analysis indicate that many ECT variables were significantly different between cases and controls at baseline, and that few measures showed significantly different changes between groups.

SNP Variation and Triglycerides – Participants examined in this study were recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at Windber Medical Center (prior to implementation of the primary Molecular Profiling Protocol described above).

We initially profiled 39 SNPs defined in recent genome-wide association studies to have an impact on CVD development or associated risk factors; we then focused on the influence of 19 SNPs on triglyceride response in the Ornish program. SNPs rs442177, rs17145738, and rs3846662 showed a significant difference in triglyceride levels by genotype at baseline and a significant change in triglycerides by genotype during the program.

Abstract Presented:

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. American Society of Human Genetics Annual Meeting, San Francisco, CA, November 2012. (Poster)
http://www.ashg.org/2012meeting/abstracts/fulltext/f120120762.htm

Abstract

Background: Triglycerides play a fundamental role in development and progression of atherosclerosis. Current guidelines advocate lifestyle change involving diet, physical activity, and weight control for management of hypertriglyceridemia patients as a first step. Recent genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) associated with plasma triglyceride levels in the general population. We hypothesize that plasma triglycerides may be influenced by genetic composition in addition to lifestyle behaviors.
Methods: We examined the influence of genetic variation on variability in triglyceride response in 178 participants who completed a prospective, non-randomized intervention designed to stabilize or reverse progression of cardiovascular disease (CVD) through dietary changes, exercise, and stress reduction. CVD risk factors were assessed at baseline, 12 weeks, and 52 weeks by standard methods. SNPs (n=19) associated with plasma triglycerides were genotyped by TaqMan ® allelic discrimination assays.
Results: Patients experienced significant improvement ($P < 0.05$) in CVD risk factors, including weight (-9%), blood pressure (-6%), total cholesterol (-7%), and triglycerides (-9%). Triglyceride response during the program differed significantly ($P < 0.05$) between genotypes for three SNPs (rs442177, rs3846662, and rs17145738) located close to the following genes: transcriptional activator AF4/FMR2 family member 1 (AFF1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), which catalyzes the rate-limiting step in cholesterol synthesis, and MLX interacting protein-like (MLXIPL), which controls transcription of genes involved in glycolysis.
Discussion: This is the first study to show that individual response to lifestyle modification for CVD risk reduction may be influenced by genetic composition. Genetic variation associated with CVD risk may provide a basis for personalized treatments to optimize cardiovascular health and requires further study.

Four additional SNPs were genotyped, 3 in close proximity to rs17145738 and one near rs3846662. Of the 4 new SNPs, 2 SNPs (rs12916 and rs714052) also showed a significant difference in triglyceride response to the Ornish program between genotypes.

All 23 SNPs have been assayed in relation to triglycerides in participants of the CRC program.

SNP Variation and Obesity – Samples (n=155) from intensive lifestyle participants have been genotyped for the following obesity-related SNPs: rs9939609, rs1421085, rs17782313, rs2815752, rs7498665, rs142433, rs925946, rs2116830, rs2241423, rs12444979, rs6548238.

Dropouts – A manuscript describing reasons why participants discontinue participation in an intensive lifestyle modification program and gender differences in attrition was completed. The reference, abstract, and key findings from the paper are provided below.

Manuscript Published:

Mamula KA, Vernalis MN, Ellsworth DL. Attrition from lifestyle modification programs for cardiovascular risk reduction: gender specific considerations and predictors. (*Under revision*)

Manuscript Abstract

Background: Identifying significant predictors of attrition from lifestyle modification programs is central to improving treatments for cardiovascular disease.
Design: Prospective clinical intervention with one year outcomes.
Methods: We examined attrition among women (n=178) and men (n=160) who enrolled in a clinical intervention designed to stabilize or reverse progression of heart disease through changes in lifestyle. Pretreatment (baseline) and initial treatment-related variables were examined separately in women and men using stepwise logistic regression to assess utility in discriminating eventual dropouts from completers.
Results: Stepwise regression models for women [$p < 0.001$, Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) = 0.772] and men ($p < 0.0001$, ROC AUC = 0.788), which best predicted dropout or completer status, contained three common variables: body mass index at entry, dietary compliance, and education level, but neither model accurately predicted attrition. Participants reported practical reasons that caused them to discontinue participation, and these factors differed between women and men: noncompliance with the program guidelines and medical/health problems were important issues for women, while work-related conflicts were most prevalent in men.
Conclusions: Clinical trials and lifestyle programs for cardiovascular risk reduction should recognize that personal barriers to continued participation differ between women and men and must strive to accommodate all barriers in order to maximize patient retention.

Key findings:
Of 338 lifestyle participants, 50 women (28.1%) and 31 men (19.4%) dropped out. The dropout rate was not significantly different between women and men (p=0.074). The majority of dropouts (39 women, 78.0%; 25 men, 80.6%) discontinued participation after the three-month examination (late dropouts), but the others dropped out before this examination and thus did not

have any outcome data. Average length of participation in the lifestyle intervention among dropouts was 23+14 weeks for women and 24+12 weeks for men.

Female late dropouts and completers, as well as male late dropouts and completers, showed significant improvement in most CVD risk factors and dietary measures at 3-months. Late dropouts showed similar progress in improving their CVD risk profile to graduates – there were no significant differences between late dropouts and completers in CVD risk factor or dietary change.

For women, stepwise regression produced a final model ($p<0.001$, ROC AUC=0.772), which best predicted status (completer or dropout), containing the following eight variables: younger age ($p=0.005$) and higher BMI ($p=0.402$) at baseline, no college attendance ($p=0.006$), living alone ($p=0.069$), non-diabetic ($p=0.318$), lack of dietary adherence during the first three months ($p=0.050$), and free from CAD ($p=0.433$) and adverse events ($p=0.052$). The model correctly classified 29.4% of female dropouts and 99.2% of completers. In men, stepwise regression produced a final model ($p<0.0001$, ROC AUC=0.788), which best predicted status, containing four variables: higher BMI ($p=0.002$) at baseline, no college attendance ($p=0.095$), employment ($p=0.025$), and lack of dietary adherence during the first three months ($p=0.010$). The model correctly classified 20.8% of male dropouts and 98.4% of completers.

Each participant who withdrew from the lifestyle program reported a primary reason for their withdrawal, or clinical staff recorded a best explanation for their discontinued participation. Among women, noncompliance with program guidelines, medical/health problems, and personal/family issues were the most frequent reasons for attrition. In men, work-related conflicts were the primary reason for attrition; noncompliance and medical/health issues were also important.

Dietary Analysis – The original objective of this project was to assess changes in dietary components during the CRC and Ornish programs. We recently expanded the focus to include vitamin supplements. During the contract period, dietary data were entered electronically and analyzed by Food Processor 10.10 software program for 85 Ornish participants at 3 time points each without vitamins (255 questionnaires) and separately with vitamins (255 questionnaires). Similarly, dietary data were entered electronically and analyzed by Food Processor 10.10 software program for 70 CRC participants at 3 time points each without vitamins (210 questionnaires) and separately with vitamins (210 questionnaires).

Food Processor results were entered into the Access database. Analysis of dietary changes was conducted for 58 dietary variables. Results from the Ornish participants showed the following: (1) significant changes in total calories (-12%), % calories from fat (-61%), and carbohydrates (+18%) during intensive lifestyle change; (2) significant changes in the following dietary components: calories from saturated fat (-74%), dietary fiber (+66%), mono-unsaturated fat (-70%), poly-unsaturated fat (-42%), trans fatty acids (-71%), cholesterol (-86%); (3) significant changes in 22 vitamins, including: A (+31%), B6 (+37%), C (+34%), and K (+81%). A low fat diet provides more minerals than a high-fat diet. An analysis of dietary trace elements showed: iron (+44% change, $P<0.001$), copper (+33% change, $P<0.001$), and manganese (+92% change, $P<0.001$) during lifestyle modification (Table 7 below).

Results from the CRC participants showed the following: (1) significant changes in total calories (-11%), % calories from fat (-15%), and carbohydrates (-8%) during moderate lifestyle change; (2) significant changes in the following dietary components: calories from saturated fat (-11%), dietary fiber (+4%), mono-unsaturated fat (-8%), poly-unsaturated fat (-8%), trans fatty acids (-3%), cholesterol (-16%); (3) significant changes in 19 vitamins, including: A (+14%), B6 (+1%), C (+11%), and K (+33%) (Table 8). Comparisons between the Ornish and the CRC program have been completed.

Table 7. 12 Week and 1 Year Change for 84 Ornish Participants and 84 Matched Controls

	Controls			**Ornish**			p-value[a]
	Baseline Mean (SD)	12 WK % Change	1 YR % Change	Baseline Mean (SD)	12 WK % Change	1 YR % Change	
Gram Weight (g)	2222.65 (755.61)	-4.5%	-6.8%	2476.15 (856.08)	-6.7%	-3.1%	0.4726
Calories (kcal)	1830.77 (562.99)	-3.2%	-6.8%	2039.19 (750.42)	-20.7%	-12.0%	0.0056
Calories from fat (kcal)	590.73 (318.2)	-3.8%	-8.3%	620.28 (367.44)	-66.9%	-61.0%	<0.0001
Calories from Sat Fat (kcal)	191 (114.55)	-2.8%	-7.9%	195.46 (132.08)	-79.1%	-73.9%	<0.0001
Protein (g)	78.03 (27.62)	-6.6%	-7.6%	83.54 (37.98)	-13.5%	-7.0%	0.3626
Carbohydrates (g)	230.87 (71.67)	-1.7%	-5.0%	276.06 (102.57)	6.7%	17.8%	<0.0001
Dietary Fiber (g)	17.68 (7.92)	0.6%	-5.3%	24.76 (12.48)	52.1%	66.4%	<0.0001
Soluble fiber (g)	1.42 (1)	9.3%	9.5%	2.01 (1.72)	77.5%	122.1%	<0.0001
Total Sugars (g)	87.91 (43.8)	-9.3%	-11.6%	95.62 (46.37)	1.7%	8.1%	0.0093
Monosaccharides (g)	12.3 (10.2)	-3.7%	-9.7%	12.32 (8.71)	56.4%	70.0%	<0.0001
Disaccharides (g)	9.32 (7.73)	-20.9%	-23.5%	12.82 (9.68)	-18.8%	-2.1%	0.2972
Other Carbs (g)	105.28 (39.06)	0.4%	-3.5%	125.95 (72.01)	3.7%	23.1%	<0.0001
Fat (g)	65.7 (35.38)	-3.8%	-8.3%	68.99 (40.89)	-66.9%	-61.0%	<0.0001
Saturated Fat (g)	21.22 (12.73)	-2.8%	-7.9%	21.72 (14.68)	-79.1%	-73.9%	<0.0001
Mono Fat (g)	15.37 (10.59)	-2.8%	-4.8%	17.21 (12.83)	-75.5%	-69.6%	<0.0001
Poly Fat (g)	8.89 (5.33)	-11.4%	-5.9%	10.61 (6.79)	-45.5%	-42.2%	0.0007
Trans Fatty Acid (g)	1.21 (1.67)	-9.8%	-8.5%	1.22 (1.53)	-82.2%	-71.0%	<0.0001
Cholesterol (mg)	234.89 (170.35)	-11.4%	-13.7%	232.78 (200.71)	-90.4%	-86.2%	<0.0001
Water (g)	1487.25 (607.48)	-0.1%	-0.5%	1679.9 (784.01)	-1.5%	-0.1%	0.9396
Vitamin A – IU	6119.05 (4860.36)	-25.2%	-5.9%	8414.5 (7811.87)	26.5%	31.0%	<0.0001
Vitamin A - RAE	544.37 (744.97)	-22.0%	-10.8%	664.9 (754.69)	3.5%	16.4%	0.0524
Carotenoid RE	391.86 (405.16)	-31.8%	-11.3%	600.06 (756.85)	43.6%	41.8%	<0.0001
Retinol RE	348.44 (721.45)	-16.5%	-10.5%	364.88 (675.87)	-29.5%	-4.5%	0.5927
Beta-Carotene (mcg)	1471.55 (2005.74)	-33.8%	-8.2%	2408.76 (3910.13)	36.3%	37.8%	0.0005
Vitamin B1 (mg)	1.26 (0.62)	0.2%	1.9%	1.43 (0.58)	25.6%	38.5%	0.0063
Vitamin B2 (mg)	1.53 (0.79)	-5.1%	1.7%	1.75 (0.69)	6.9%	18.8%	0.3429

Vitamin B3 (mg)	18.7 (8.74)	-0.2%	-5.3%	20.9 (10.5)	-12.3%	2.5%	0.1307
Vitamin B3 - Niacin (mg)	26.03 (11.21)	-2.9%	-6.8%	29.32 (15.8)	-18.1%	-5.7%	0.2041
Vitamin B6 (mg)	1.47 (1.08)	-0.1%	-2.5%	1.71 (0.83)	19.5%	37.0%	0.0434
Vitamin B12 (mcg)	3.71 (4.22)	-3.9%	-1.8%	4.01 (3.21)	-13.8%	4.9%	0.1981
Biotin (mcg)	14.35 (14.65)	-23.1%	-12.6%	15.82 (14.75)	23.9%	23.9%	0.0002
Vitamin C (mg)	100.56 (70.88)	-16.1%	-13.0%	109.31 (90.15)	35.9%	34.2%	<0.0001
Vitamin D – IU	91.03 (93.57)	29.0%	20.5%	112.16 (82.64)	-38.4%	-1.2%	0.0001
Vitamin D (mcg)	2.26 (2.35)	29.2%	21.2%	2.77 (2.07)	-37.7%	-0.4%	0.0001
Vitamin E – Alpha (mg)	4.2 (3.87)	-6.7%	-7.0%	4.87 (3.97)	-10.6%	2.8%	0.1836
Folate (mcg)	329.89 (217.28)	-7.2%	-3.6%	381.3 (258.54)	24.8%	37.5%	0.0004
Folate DFE (mcg)	409.79 (343.15)	-11.8%	-2.6%	442.89 (209.61)	37.7%	52.1%	0.0002
Vitamin K (mcg)	47.04 (67.4)	-42.3%	-26.8%	48.89 (50.78)	44.9%	80.6%	0.0051
Pantothenic Acid (mg)	3.31 (1.56)	7.5%	9.6%	4.12 (2.02)	5.9%	3.6%	0.9263
Calcium (mg)	775.84 (598.84)	-10.9%	-6.3%	848.29 (359.63)	16.7%	22.7%	0.0024
Chromium (mcg)	4.4 (6.03)	-15.7%	-16.3%	3.85 (5.02)	-1.8%	40.5%	0.0724
Copper (mg)	0.77 (0.38)	-2.2%	7.2%	1.04 (0.62)	19.5%	32.6%	0.0005
Fluoride (mg)	0.84 (0.99)	-12.9%	1.0%	0.82 (0.79)	-56.1%	-61.8%	<0.0001
Iodine (mcg)	30.88 (30.52)	-26.6%	-14.4%	39.01 (35.8)	-22.1%	-19.2%	0.8876
Iron (mg)	16.58 (8.46)	-4.3%	-0.5%	18.36 (7.2)	30.1%	44.0%	<0.0001
Magnesium (mg)	200.47 (93.12)	-2.9%	-4.0%	232.8 (85.82)	32.9%	51.9%	<0.0001
Manganese (mg)	2.36 (1.23)	2.6%	0.8%	3.17 (1.98)	75.0%	92.4%	<0.0001
Molybdenum (mcg)	8.92 (8.36)	-17.4%	3.1%	12.31 (13.29)	-19.8%	57.6%	0.8009
Phosphorus (mg)	832.79 (355.58)	-6.4%	-7.2%	938.59 (413.74)	4.6%	15.8%	0.0148
Potassium (mg)	1998.21 (777.15)	-2.2%	0.2%	2377.16 (904.93)	10.2%	21.3%	0.0013
Selenium (mcg)	70.27 (37.98)	-12.3%	1.8%	79.96 (59.28)	-9.8%	-2.7%	0.4970
Sodium (mg)	3128.05 (1231.74)	-2.6%	-7.4%	3378.48 (1558.59)	-12.4%	-8.3%	0.8366
Zinc (mg)	7.45 (4.07)	2.8%	1.7%	9.64 (4.9)	-0.9%	4.7%	0.8259
Omega 3 Fatty Acid (g)	0.94 (0.66)	-18.6%	-12.2%	1.06 (0.84)	-59.2%	-56.3%	<0.0001
Omega 6 Fatty Acid (g)	7.18 (4.85)	-15.4%	-8.7%	8.6 (6)	-52.8%	-47.9%	0.0001
Alcohol (g)	4.81 (9.49)	9.2%	-17.5%	2.12 (7.29)	-53.0%	-25.9%	0.2871
Caffeine (mg)	127.24 (127.1)	-3.4%	15.5%	101.6 (156.85)	-93.2%	-91.7%	<0.0001
Choline (mg)	155.18 (104.48)	-12.8%	-5.6%	168.44 (93.98)	-22.6%	-10.1%	0.4298

[a] p-value represents difference in change over time between Ornish and Control groups based on repeated measures ANOVA with time and group as within-subject factors

Table 8. 12 Week and 1 Year Change for 70 Matched Ornish/CRC/Control Subjects

	Controls			CRC			p-value[a]
	Baseline Mean (SD)	% Change from BL	% Change from BL	Baseline Mean (SD)	% Change from BL	% Change from BL	
Gram Weight (g)	2223.88 (746.96)	-5.0%	-7.2%	2342.3 (995.81)	-1.7%	-3.6%	0.6768
Calories (kcal)	1849.05 (576.35)	-3.0%	-6.79%	1859.95 (638.75)	-11.1%	-10.7%	0.0290
Calories from fat (kcal)	604.35 (332.88)	-3.9%	-9.38%	596.24 (286.91)	-19.6%	-14.8%	<0.0001
Calories from Sat Fat (kcal)	196.83 (120.5)	-3.1%	-9.2%	183.66 (84.47)	-16.6%	-10.7%	<0.0001
Protein (g)	79.33 (28.7)	-6.6%	-9.8%	79.45 (28.43)	-4.7%	-6.9%	0.5020
Carbohydrates (g)	232.95 (71.4)	-1.7%	-3.3%	237.86 (85.66)	-7.0%	-8.1%	<0.0001
Dietary Fiber (g)	18.15 (8.32)	0.1%	-6.1%	22.22 (10.12)	8.4%	3.9%	<0.0001
Soluble fiber (g)	1.48 (1.06)	6.4%	2.9%	1.56 (1.37)	5.2%	10.8%	<0.0001
Total Sugars (g)	88.67 (43.17)	-9.2%	-10.2%	81.21 (33.6)	2.9%	-1.0%	0.0302
Monosaccharides (g)	12.5 (10.27)	-2.3%	-17.7%	11.11 (9.89)	26.9%	22.5%	<0.0001
Disaccharides (g)	9.59 (7.96)	-17.8%	-26.9%	9.01 (9)	-7.8%	5.5%	0.2092
Other Carbs (g)	104.85 (39.66)	1.5%	-0.9%	113.95 (53.17)	-16.6%	-14.7%	<0.0001
Fat (g)	67.23 (37.01)	-3.9%	-9.4%	66.35 (31.91)	-19.6%	-14.8%	<0.0001
Saturated Fat (g)	21.87 (13.39)	-3.1%	-9.2%	20.42 (9.38)	-16.6%	-10.8%	<0.0001
Mono Fat (g)	16.07 (10.9)	-7.1%	-8.7%	12.87 (8.43)	-6.0%	-8.1%	<0.0001
Poly Fat (g)	9.49 (5.42)	-15.2%	-12.2%	7.78 (5.31)	-10.2%	-7.9%	0.0244
Trans Fatty Acid (g)	1.28 (1.78)	-6.4%	-16.0%	1 (1.13)	-40.6%	-3.4%	<0.0001
Cholesterol (mg)	245.31 (179.29)	-11.9%	-16.8%	235.1 (142.71)	-15.1%	-15.8%	<0.0001
Water (g)	1475.76 (600.49)	-4.5%	-4.1%	1592.41 (854.82)	-0.2%	-0.2%	0.8560
Vitamin A – IU	8371.96 (6605.64)	-19.3%	-5.3%	8326.53 (5208.91)	30.6%	13.7%	0.0002
Vitamin A – RAE	930.26 (888.15)	-11.5%	-1.4%	854.84 (450.14)	8.1%	2.9%	0.0045
Carotenoid RE	465.28 (443.62)	-30.3%	-16.4%	419.71 (412.68)	53.5%	26.0%	<0.0001
Retinol RE	697.63 (833)	-5.2%	3.7%	645.78 (376.96)	-6.8%	-4.7%	0.5434
Beta-Carotene (mcg)	1884.96 (2232.09)	-29.0%	-15.3%	1652.39 (2074.98)	77.2%	25.9%	0.0026
Vitamin B1 (mg)	1.96 (0.94)	2.1%	6.5%	1.99 (1.21)	-6.4%	-1.8%	0.0525
Vitamin B2 (mg)	2.33 (1.13)	-1.9%	7.3%	2.3 (1.18)	-2.0%	3.6%	0.7066
Vitamin B3 (mg)	28.21 (12.88)	2.3%	0.0%	28.91 (15.15)	-4.8%	-5.3%	0.3531

Vitamin B3 - Niacin (mg)	78.65 (224.28)	-0.1%	25.6%	42.88 (59.82)	-2.7%	-3.6%	0.4385
Vitamin B6 (mg)	4.27 (12.32)	2.8%	4.6%	4.53 (12.15)	0.5%	1.4%	0.0058
Vitamin B12 (mcg)	15.07 (12.49)	6.8%	11.1%	16.5 (12.96)	-6.7%	-2.4%	0.5675
Biotin (mcg)	28.53 (22.68)	-7.9%	0.6%	29.83 (22.41)	14.6%	19.2%	<0.0001
Vitamin C (mg)	195.52 (195.77)	-3.1%	7.5%	192.14 (188.55)	11.0%	11.3%	0.0110
Vitamin D – IU	277.75 (228.71)	13.1%	16.9%	319.69 (222.22)	-1.4%	-3.4%	0.0568
Vitamin D (mcg)	6.98 (5.72)	13.0%	17.9%	8.33 (5.86)	-3.0%	9.6%	0.1982
Vitamin E - Alpha (mg)	66 (134.29)	3.3%	54.4%	19.08 (34.97)	-0.2%	0.6%	0.4832
Folate (mcg)	507.56 (261.89)	0.4%	6.7%	526.95 (312.17)	-3.5%	-2.8%	<0.0001
Folate DFE (mcg)	708.72 (417.98)	-0.8%	10%	747.47 (481.58)	-7.8%	-5.7%	<0.0001
Vitamin K (mcg)	54.69 (72.96)	-38.5%	-29.8%	48.43 (68.54)	27.2%	32.6%	0.0496
Pantothenic Acid (mg)	7.99 (5.27)	6.2%	11.5%	8.43 (5.79)	-1.9%	5.2%	0.7218
Calcium (mg)	1112.77 (733.2)	-9.9%	-1.9%	1115.3 (565.36)	6.9%	3.1%	0.0013
Chromium (mcg)	72.54 (76.45)	7.5%	13.7%	84.85 (76.09)	-10.0%	-1.4%	0.0916
Copper (mg)	1.71 (1.1)	2.6%	9.8%	1.76 (1.13)	0.0%	3.7%	<0.0001
Fluoride (mg)	0.87 (1.06)	-13.1%	1.6%	0.67 (0.79)	-3.4%	-1.7%	<0.0001
Iodine (mcg)	100.3 (83.75)	-1.4%	5.1%	110.82 (83.31)	-5.6%	5.9%	0.6763
Iron (mg)	16.64 (8.49)	-5.2%	2.0%	16.59 (10.29)	5.5%	5.4%	<0.0001
Magnesium (mg)	270.9 (174.68)	-1.7%	-2.7%	253.52 (126.59)	3.9%	2.6%	<0.0001
Manganese (mg)	3.37 (1.72)	-1.7%	2.3%	3.3 (1.94)	17.4%	10.0%	<0.0001
Molybdenum (mcg)	43.1 (39.98)	3.4%	9.1%	49.76 (43.79)	-4.2%	5.9%	0.8058
Phosphorus (mg)	876.63 (353.88)	-7.4%	-11.0%	812.87 (356.57)	-4.7%	-0.7%	0.0232
Potassium (mg)	2082.41 (780.65)	-3.8%	-3.3%	1820.53 (833.08)	8.8%	8.9%	0.0096
Selenium (mcg)	84.05 (47.89)	-9.9%	0.8%	74.21 (39.36)	0.5%	0.1%	0.8793
Sodium (mg)	3193.83 (1275.34)	-3.3%	-6.2%	3191.48 (1466.46)	-14.5%	-9.8%	0.9094
Zinc (mg)	18.23 (25.64)	3.1%	14.1%	15.11 (8.9)	-7.3%	-0.1%	0.1357
Omega 3 Fatty Acid (g)	147.64 (299.97)	16.0%	10.3%	148.74 (472.31)	0.1%	-11.5%	0.2966
Omega 6 Fatty Acid (g)	7.51 (5)	-19.2%	-14.5%	5.68 (4.48)	-9.7%	-9.1%	<0.0001
Alcohol (g)	3.87 (8.42)	22.8%	-27.8%	4.03 (9.12)	-5.1%	-26.3%	0.6849
Caffeine (mg)	125.35 (122.83)	-6.7%	17.1%	81.82 (104.97)	4.8%	-7.5%	<0.0001
Choline (mg)	161.74 (107.62)	-13.5%	-13.0%	144.18 (79.03)	3.2%	2.3%	0.1154

[a] p-value based on repeated measures ANOVA with time and group as within-subjects factors

CRC Program Status:
Enrollment in this program ended June 30, 2013. Continuing review of the protocol was approved by the Chesapeake IRB on 15 Jan 2015 (08-07).

Subject Enrollment and Demographics:
Demographic characteristics of participants were: average age 58.9 years, 58% female, 22% veterans or the spouse of a veteran, and 20% with diagnosed coronary heart disease. Total subject enrollment was 264 (144 intervention; 120 controls); 59 drop-outs; 34 control participants transferred to the intervention arm after one year as a control. A summary of final patient progress is as follows:
- 124 intervention participants completed the intervention (4-6 months); 102 completed first 6th month follow-up time point; 68 completed year 1; 45 reached month 18; 26 reached year 2; 14 completed Month 30; 11 have completed year 3.
- 91 controls completed the "waiting period complete" time point (6 months); 66 completed the first 6th month follow-up time point; 29 reached year 1; 21 completed month 18; 11 reached year 2; 8 have completed year 3.
- 90 intervention participant satisfaction surveys were completed and returned

Clinical activities conducted during the contract period include:
- 1566 total visits including periodic follow up phone calls were made to the intervention arm participants
- 196 total visits including periodic follow up phone calls were made to the control arm participants

Outcome Data
A substantial portion of CRC participants had clinically-relevant disorders at baseline: 61% hypertensive, 42% were obese, 58% had high cholesterol. Weight/BMI (-2.3%), blood pressure (-5.1%), and most psychometric measures improved significantly by the end of the intervention (Table 9A).

Results from the first long-term follow up time point (6 months after completion of the intervention) are shown in Table 9B. Over the course of approximately 8-10 months, weight, BMI, total cholesterol, triglycerides, blood pressure, and the psychometric measures maintained statistical significance proving that the positive improvements in these traditional risk factors for CAD can be maintained over a longer period of time.

In Table 9C, results 1 year after completion of the intervention are shown. Weight, BMI, blood pressure, CIMT measurements as well as psychosocial and sleep factors continued to maintain statistical significance. Most variables continued to trend in the desired direction.

Tables 9D and 9E show the furthest time points reached before the program was stopped, 18 months and 2 years respectively after completion of the intervention. Although a relatively small sample size, risk factors continued to show positive improvements.

Table 9A. Comparison of "Baseline" to "Intervention Complete" (4-6 months) data for participants in the intervention arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Intervention Complete Value (SD)	Average Change	P Value
Weight (lbs.)	124	195.79 (44.0)	191.21 (41.6)	-4.6	<0.00001
Body Mass Index	124	31.33 (6.1)	30.58 (6.1)	-0.8	<0.00001
Total Cholesterol (mg/dl)	122	187.45 (39.3)	184.91 (36.7)	-2.5	0.3227
High Density Lipids (mg/dl)	122	46.62 (12.1)	47.48 (11.0)	0.9	0.1921
Low Density Lipids (mg/dl)	122	113.98 (31.8)	112.70 (30.5)	-1.3	0.5574
Triglycerides (mg/dl)	122	135.77 (68.9)	123.17 (54.0)	-12.6	<0.01
Systolic Blood Pressure	122	131.79 (17.0)	126.10 (16.5)	-5.7	<0.0001
Diastolic Blood Pressure	122	80.41 (11.3)	76.31 (9.9)	-4.1	<0.0001
Depression Scale [CES-D]	121	10.46 (9.5)	7.41 (7.9)	-3.0	<0.00001
Hostility Scale [Cook-Medley]	121	6.93 (4.5)	6.07 (4.3)	-0.9	<0.001
Perceived Stress Scale [PSS]	121	13.48 (6.2)	10.78 (5.5)	-2.7	<0.00001
Daily Total Fat (grams)	119	67.28 (32.0)	54.47 (23.7)	-12.8	<0.0001
Daily Saturated Fat (grams)	119	21.41 (10.9)	17.13 (8.5)	-4.3	<0.001
Avg. CCA/Mean IMT	121	0.716 (0.1669)	0.683 (0.1401)	-0.032	<0.0001
Avg. CCA / Max IMT	121	0.825 (0.1944)	0.782 (0.1523)	0.04	<0.00001
Fasting Glucose (mg/dl)	122	103 (30.5)	102 (23.9)	-1.7	0.4385
HgbA1c	122	5.9 (0.98)	5.9 (0.98)	0.0	0.7947
Cortisol	121	11.3 (3.98)	12.9 (4.06)	1.5	<0.0001
TSH	122	2.02 (1.028)	2.14 (1.224)	0.1	0.2330
Epworth Sleepiness Scale	120	9 (4.4)	7 (4.0)	-1.2	<0.0001
Pittsburgh Sleep Quality Index	120	7 (4.0)	6 (3.6)	-1.2	<0.00001

Table 9B. Change in outcome variables 6 months after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Month 6 Value (SD)	Average Change	P Value
Weight (lbs.)	98	195.27 (46.0)	190.23 (43.6)	-5.0	<0.00001
Body Mass Index	98	31.18 (6.3)	30.50 (6.4)	-0.7	<0.001
Total Cholesterol (mg/dl)	99	186.54 (38.7)	180.33 (37.3)	-6.2	<0.05
High Density Lipids (mg/dl)	99	47.18 (12.3)	47.64 (12.2)	0.5	0.5307
Low Density Lipids (mg/dl)	99	112.26 (31.2)	108.24 (31.7)	-4.0	0.1444
Triglycerides (mg/dl)	99	137.30 (69.1)	122.89 (61.7)	-14.4	<0.01
Systolic Blood Pressure	98	131.65 (17.5)	127.02 (17.3)	-4.6	<0.01
Diastolic Blood Pressure	98	80.14 (11.4)	75.59 (9.2)	-4.6	<0.001
Depression Scale [CES-D]	97	11.08 (10.1)	8.30 (10.1)	-2.8	<0.0001
Hostility Scale [Cook-Medley]	97	7.10 (4.5)	6.52 (4.4)	-0.6	0.0603
Perceived Stress Scale [PSS]	97	13.62 (6.5)	11.36 (6.9)	-2.3	<0.0001
Daily Total Fat (grams)	87	65.74 (32.3)	55.79 (22.5)	-10.0	<0.01
Daily Saturated Fat (grams)	87	20.69 (10.7)	17.89 (9.2)	-2.8	<0.05

Avg. CCA/Mean IMT	97	0.730 (0.1570)	0.684 (0.1452)	-0.046	<0.00001
Avg. CCA / Max IMT	97	0.841 (0.1804)	0.778 (0.1592)	-0.1	<0.00001
Fasting Glucose (mg/dl)	100	105 (31.6)	106 (28.6)	0.6	0.7865
HgbA1c	99	6.0 (1.02)	6.0 (1.20)	-0.1	0.1569
Cortisol	96	11.6 (3.78)	12.5 (3.96)	0.9	0.0516
TSH	99	2.04 (1.048)	2.25 (1.343)	0.2	<0.05
Epworth Sleepiness Scale	97	9 (4.5)	8 (5.1)	-1.0	<0.01
Pittsburgh Sleep Quality Index	97	8 (4.1)	6 (3.7)	-1.2	<0.001

Table 9C. Change in outcome variables 1 year after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	64	192.30 (44.5)	185.01 (40.1)	-7.3	<0.0001
Body Mass Index	64	30.46 (6.0)	29.31 (5.2)	-1.1	<0.0001
Total Cholesterol (mg/dl)	64	185.73 (39.9)	182.06 (39.6)	-3.7	0.3474
High Density Lipids (mg/dl)	64	49.08 (13.7)	49.17 (12.7)	0.1	0.9338
Low Density Lipids (mg/dl)	64	110.13 (31.1)	109.89 (33.3)	-0.2	0.9398
Triglycerides (mg/dl)	64	133.78 (72.9)	115.13 (49.8)	-18.7	<0.01
Systolic Blood Pressure	64	131.22 (16.0)	125.88 (16.0)	-5.3	<0.01
Diastolic Blood Pressure	64	80.97 (11.3)	76.19 (7.8)	-4.8	<0.001
Depression Scale [CES-D]	63	9.95 (10.2)	7.30 (7.6)	-2.7	<0.01
Hostility Scale [Cook-Medley]	63	7.29 (4.7)	6.63 (4.6)	-0.7	0.0727
Perceived Stress Scale [PSS]	63	13.02 (6.5)	10.51 (5.7)	-2.5	<0.0001
Daily Total Fat (grams)	58	65.44 (33.8)	56.77 (27.3)	-8.7	0.0894
Daily Saturated Fat (grams)	58	20.06 (10.7)	18.54 (10.7)	-1.5	0.3736
Avg. CCA/Mean IMT	64	0.753 (0.1564)	0.675 (0.1307)	-0.078	<0.00001
Avg. CCA / Max IMT	64	0.869 (0.1800)	0.772 (0.1502)	-0.1	<0.00001
Fasting Glucose (mg/dl)	64	105 (34.8)	106 (36.1)	1.2	0.5614
HgbA1c	64	6.1 (1.09)	6.0 (1.24)	-0.2	<0.001
Cortisol	64	11.6 (4.08)	13.1 (4.04)	1.4	<0.01
TSH	64	1.97 (1.097)	2.19 (1.099)	0.2	0.0922
Epworth Sleepiness Scale	63	9 (4.6)	7 (4.0)	-1.8	<0.0001
Pittsburgh Sleep Quality Index	63	8 (4.6)	6 (4.0)	-1.9	<0.0001

Table 9D. Change in outcome variables 18 months after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Month 18 Value (SD)	Average Change	P Value
Weight (lbs.)	45	186.11 (42.1)	179.79 (39.2)	-6.3	<0.00001
Body Mass Index	45	29.67 (5.4)	28.53 (5.0)	-1.1	<0.00001
Total Cholesterol (mg/dl)	45	192.09 (43.5)	184.78 (39.8)	-7.3	0.1162
High Density Lipids (mg/dl)	45	51.29 (14.2)	49.84 (11.8)	-1.4	0.2484

Low Density Lipids (mg/dl)	45	114.84 (33.5)	112.87 (31.3)	-2.0	0.6123
Triglycerides (mg/dl)	45	131.31 (63.4)	110.40 (42.4)	-20.9	<0.01
Systolic Blood Pressure	45	130.40 (15.1)	128.44 (17.8)	-2.0	0.3854
Diastolic Blood Pressure	45	80.27 (11.5)	75.24 (9.9)	-5.0	<0.01
Depression Scale [CES-D]	44	8.70 (10.0)	8.61 (10.7)	-0.1	0.9472
Hostility Scale [Cook-Medley]	44	6.82 (4.7)	6.32 (5.3)	-0.5	0.3776
Perceived Stress Scale [PSS]	44	12.16 (6.7)	10.93 (7.4)	-1.2	0.2256
Daily Total Fat (grams)	39	66.15 (32.1)	54.24 (33.7)	-11.9	0.1080
Daily Saturated Fat (grams)	39	19.91 (10.2)	19.56 (17.3)	-0.4	0.9143
Avg. CCA/Mean IMT	42	0.788 (0.1478)	0.648 (0.1316)	-0.139	<0.00001
Avg. CCA / Max IMT	42	0.910 (0.1748)	0.731 (0.1485)	-0.2	<0.00001
Fasting Glucose (mg/dl)	45	98 (13.4)	103 (30.7)	4.8	0.1890
HgbA1c	45	6.0 (0.92)	6.0 (1.26)	-0.1	0.3545
Cortisol	45	11.8 (4.24)	12.8 (4.17)	1.0	0.1440
TSH	45	2.11 (1.219)	2.35 (1.915)	0.2	0.2913
Epworth Sleepiness Scale	44	8 (4.8)	7 (4.7)	-1.2	<0.05
Pittsburgh Sleep Quality Index	44	8 (4.7)	6 (3.3)	-2.1	<0.0001

Table 9E. Change in outcome variables 2 years after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Year 2 Value (SD)	Average Change	P Value
Weight (lbs.)	26	191.57 (42.5)	185.08 (40.1)	-6.5	<0.001
Body Mass Index	26	30.17 (5.7)	28.98 (5.2)	-1.2	<0.0001
Total Cholesterol (mg/dl)	26	187.73 (41.2)	178.15 (44.1)	-9.6	0.2125
High Density Lipids (mg/dl)	26	52.96 (15.6)	51.38 (17.4)	-1.6	0.3900
Low Density Lipids (mg/dl)	26	110.12 (30.5)	106.00 (32.2)	-4.1	0.4899
Triglycerides (mg/dl)	26	126.19 (67.4)	104.12 (54.5)	-22.1	<0.05
Systolic Blood Pressure	26	131.38 (14.6)	128.92 (15.9)	-2.5	0.4549
Diastolic Blood Pressure	26	79.15 (10.4)	74.77 (7.8)	-4.4	<0.05
Depression Scale [CES-D]	25	10.28 (9.8)	7.56 (8.1)	-2.7	0.0843
Hostility Scale [Cook-Medley]	25	7.00 (4.3)	5.56 (3.9)	-1.4	<0.05
Perceived Stress Scale [PSS]	25	12.00 (6.9)	9.48 (6.3)	-2.5	<0.05
Daily Total Fat (grams)	17	61.03 (29.4)	62.65 (18.6)	1.6	0.8461
Daily Saturated Fat (grams)	17	19.73 (11.9)	19.80 (8.8)	0.1	0.9850
Avg. CCA/Mean IMT	26	0.859 (0.1254)	0.662 (0.1244)	-0.197	<0.00001
Avg. CCA / Max IMT	26	0.995 (0.1514)	0.754 (0.1375)	-0.2	<0.00001
Fasting Glucose (mg/dl)	26	101 (13.7)	105 (28.9)	4.1	0.3312
HgbA1c	26	6.2 (1.05)	5.9 (1.30)	-0.3	<0.05
Cortisol	26	12.2 (4.42)	12.7 (3.70)	0.4	0.6807
TSH	26	2.28 (1.429)	2.51 (2.020)	0.2	0.4380
Epworth Sleepiness Scale	25	8 (4.5)	6 (4.4)	-1.5	0.0990
Pittsburgh Sleep Quality Index	25	9 (5.1)	7 (3.6)	-2.1	<0.05

In subjects randomized to the control arm of the study, who did not participate in the lifestyle change intervention showed no significant changes in risk factors, except for CIMT at the Waiting Period Complete time point (Table 10A). Subsequent follow up time points (Table 10B: 6 month time point; Table 10C: 1 year; Table 10D: 18 months) continued to show that most risk factors did not change significantly in controls. This lack of consistent improvement within the control arm further proved the benefits of a team-base, patient-centered lifestyle change model in improving risk for developing heart disease.

Table 10A. Change in outcome variables from baseline to "waiting period complete" time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P Value
Weight (lbs.)	90	189.48 (44.7)	189.13 (44.1)	-0.3	0.6810
Body Mass Index	90	30.99 (7.2)	30.85 (6.9)	-0.1	0.5765
Total Cholesterol (mg/dl)	91	191.26 (36.6)	189.05 (36.2)	-2.2	0.4477
High Density Lipids (mg/dl)	91	49.82 (14.9)	49.43 (13.1)	-0.4	0.5752
Low Density Lipids (mg/dl)	91	115.93 (30.6)	113.26 (30.9)	-2.7	0.3159
Triglycerides (mg/dl)	91	129.58 (61.1)	130.46 (62.9)	0.9	0.8525
Systolic Blood Pressure	91	128.48 (18.0)	130.04 (20.0)	1.6	0.3707
Diastolic Blood Pressure	91	78.42 (10.1)	78.84 (9.9)	0.4	0.6771
Depression Scale [CES-D]	88	11.35 (9.8)	10.26 (8.7)	-1.1	0.1368
Hostility Scale [Cook-Medley]	88	7.30 (4.7)	6.89 (4.8)	-0.4	0.1879
Perceived Stress Scale [PSS]	88	13.03 (7.4)	12.57 (7.5)	-0.5	0.3681
Daily Total Fat (grams)	78	70.74 (30.3)	70.38 (33.4)	-0.4	0.9338
Daily Saturated Fat (grams)	78	22.27 (10.3)	22.35 (13.0)	0.1	0.9651
Avg. CCA/Mean IMT	89	0.740 (0.2148)	0.705 (0.1820)	-0.035	<0.01
Avg. CCA / Max IMT	89	0.852 (0.2514)	0.803 (0.2039)	0.0	<0.01
Fasting Glucose (mg/dl)	91	106 (34.1)	106 (38.0)	0.1	0.9492
HgbA1c	90	5.9 (1.22)	5.9 (1.02)	0.0	0.8935
Cortisol	91	12.4 (4.74)	13.2 (4.65)	0.8	0.0656
TSH	89	1.97 (1.160)	2.28 (2.127)	0.3	0.1832
Epworth Sleepiness Scale	88	8 (4.4)	7 (4.0)	-0.5	0.1302
Pittsburgh Sleep Quality Index	88	7 (3.8)	6 (3.7)	-0.4	0.2841

Table 10B. Change in outcome variables at the 6 month time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Month 6 Value (SD)	Average Change	P Value
Weight (lbs.)	63	189.09 (42.8)	189.48 (44.4)	0.4	0.7620
Body Mass Index	62	31.05 (6.4)	30.61 (6.5)	-0.4	0.1388
Total Cholesterol (mg/dl)	63	190.60 (36.2)	182.25 (33.9)	-8.3	<0.05
High Density Lipids (mg/dl)	63	49.27 (14.3)	48.57 (16.1)	-0.7	0.6364
Low Density Lipids (mg/dl)	63	115.83 (29.9)	109.76 (28.4)	-6.1	<0.05
Triglycerides (mg/dl)	63	130.51 (62.1)	126.16 (54.5)	-4.3	0.5203

Systolic Blood Pressure	63	129.14 (18.1)	129.08 (23.5)	-0.1	0.9784
Diastolic Blood Pressure	63	78.79 (10.6)	77.56 (10.9)	-1.2	0.3813
Depression Scale [CES-D]	61	11.98 (10.4)	11.13 (9.4)	-0.9	0.4464
Hostility Scale [Cook-Medley]	61	7.70 (5.0)	7.39 (4.9)	-0.3	0.3408
Perceived Stress Scale [PSS]	61	13.64 (7.3)	12.82 (7.0)	-0.8	0.2689
Daily Total Fat (grams)	59	71.28 (32.4)	64.22 (26.5)	-7.1	0.1423
Daily Saturated Fat (grams)	59	22.33 (10.8)	21.75 (10.0)	-0.6	0.7226
Avg. CCA/Mean IMT	61	0.788 (0.2135)	0.711 (0.1813)	-0.076	<0.0001
Avg. CCA / Max IMT	61	0.906 (0.2504)	0.821 (0.2030)	-0.1	<0.001
Fasting Glucose (mg/dl)	63	109 (38.6)	109 (38.9)	-0.6	0.8266
HgbA1c	63	6.0 (1.36)	6.0 (1.14)	-0.1	0.4075
Cortisol	63	12.4 (4.71)	13.2 (4.49)	0.8	0.1303
TSH	63	1.96 (1.267)	2.09 (0.984)	0.1	0.3602
Epworth Sleepiness Scale	61	8 (4.4)	8 (4.1)	-0.5	0.2192
Pittsburgh Sleep Quality Index	61	7 (3.7)	7 (3.8)	0.0	0.8992

Table 10C. Change in outcome variables at year 1 time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	25	196.02 (42.7)	192.26 (43.0)	-3.8	0.0940
Body Mass Index	25	31.18 (6.1)	30.39 (6.2)	-0.8	0.0639
Total Cholesterol (mg/dl)	25	200.00 (37.2)	184.56 (38.8)	-15.4	<0.05
High Density Lipids (mg/dl)	25	54.24 (18.0)	49.64 (16.6)	-4.6	<0.01
Low Density Lipids (mg/dl)	25	120.48 (31.4)	111.12 (31.7)	-9.4	0.1498
Triglycerides (mg/dl)	25	133.60 (77.6)	118.56 (71.9)	-15.0	0.1146
Systolic Blood Pressure	25	131.28 (15.4)	128.96 (18.3)	-2.3	0.4832
Diastolic Blood Pressure	25	80.16 (9.2)	77.92 (11.8)	-2.2	0.2522
Depression Scale [CES-D]	25	10.32 (9.6)	9.88 (10.1)	-0.4	0.6818
Hostility Scale [Cook-Medley]	25	7.88 (5.5)	7.72 (4.5)	-0.2	0.7954
Perceived Stress Scale [PSS]	25	11.84 (8.0)	12.12 (6.8)	0.3	0.7855
Daily Total Fat (grams)	23	79.23 (39.6)	70.68 (33.6)	-8.5	0.2531
Daily Saturated Fat (grams)	23	23.83 (12.2)	23.66 (11.8)	-0.2	0.9455
Avg. CCA/Mean IMT	24	0.895 (0.1728)	0.757 (0.1433)	-0.138	<0.00001
Avg. CCA / Max IMT	24	1.031 (0.1885)	0.863 (0.1570)	-0.2	<0.00001
Fasting Glucose (mg/dl)	25	117 (43.7)	109 (43.5)	-8.0	0.2006
HgbA1c	25	6.3 (1.36)	6.0 (0.94)	-0.3	0.0657
Cortisol	25	13.3 (4.21)	12.9 (4.33)	-0.4	0.7046
TSH	25	1.94 (1.227)	2.04 (0.784)	0.1	0.5320
Epworth Sleepiness Scale	25	8 (4.6)	7 (4.0)	-0.8	0.2612
Pittsburgh Sleep Quality Index	25	7 (3.9)	6 (3.4)	-0.5	0.3153

Table 10D. Change in outcome variables 18 month time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Month 18 Value (SD)	Average Change	P Value
Weight (lbs.)	21	199.05 (39.5)	195.55 (44.7)	-3.5	0.3697
Body Mass Index	21	31.83 (5.8)	30.86 (6.5)	-1.0	0.1862
Total Cholesterol (mg/dl)	21	200.33 (38.0)	192.67 (45.8)	-7.7	0.3567
High Density Lipids (mg/dl)	21	53.86 (19.3)	52.14 (17.7)	-1.7	0.3017
Low Density Lipids (mg/dl)	21	121.14 (32.9)	116.05 (37.2)	-5.1	0.5624
Triglycerides (mg/dl)	21	132.71 (73.5)	122.24 (81.9)	-10.5	0.3635
Systolic Blood Pressure	17	133.76 (14.2)	128.94 (20.8)	-4.8	0.3726
Diastolic Blood Pressure	17	78.82 (9.3)	78.94 (9.5)	0.1	0.9550
Depression Scale [CES-D]	22	8.91 (6.6)	8.95 (7.1)	0.0	0.9658
Hostility Scale [Cook-Medley]	22	7.50 (4.7)	7.05 (4.9)	-0.5	0.5167
Perceived Stress Scale [PSS]	22	10.64 (6.6)	10.68 (6.1)	0.0	0.9690
Daily Total Fat (grams)	19	74.54 (38.2)	78.79 (30.3)	4.2	0.6458
Daily Saturated Fat (grams)	19	22.26 (11.7)	25.56 (9.1)	3.3	0.1852
Avg. CCA/Mean IMT	21	0.902 (0.1589)	0.729 (0.1409)	-0.174	<0.00001
Avg. CCA / Max IMT	21	1.037 (0.1808)	0.825 (0.1612)	-0.2	<0.00001
Fasting Glucose (mg/dl)	21	115 (36.9)	108 (22.8)	-6.9	0.1994
HgbA1c	21	6.3 (1.24)	6.0 (0.93)	-0.3	0.1391
Cortisol	21	13.4 (4.24)	11.6 (3.93)	-1.8	0.1292
TSH	21	2.09 (1.273)	2.44 (1.217)	0.3	<0.05
Epworth Sleepiness Scale	22	8 (4.2)	8 (3.6)	-0.1	0.8525
Pittsburgh Sleep Quality Index	22	6 (3.8)	6 (3.6)	-0.2	0.8005

Abstract Presented:

Burke A, Ellsworth DL, Haberkorn MJ, Lechak F, Sullivan J, Adams B, Patney HL, Mamula KA, Vernalis MN, Kashani M. Coaching patients to control hypertension through a team-based, patient-centered program: the Cardiovascular Risk Clinic. *J Cardiovasc Nurs* 2013; 28(4):309A.

Preventive Cardiovascular Nurses Association 19th Annual Symposium, Las Vegas, NV, May 2013. (Poster **- 1st Place Winner: Innovation in Patient Care Category**)

Abstract

Background: Hypertension (HTN) is a major risk factor of cardiovascular disease and stroke. Although treatment is associated with reductions in the incidence of these diseases, nearly one-third of hypertensive adults remain uncontrolled.

Purpose: The Cardiovascular Risk Clinic, a multidisciplinary risk factor modification program, empowers participants with the skills needed to control HTN.

Design: Phase 1 of the intervention included a physician led cardiovascular disease risk assessment, followed by monthly individual appointments during a 4-month period with an exercise physiologist, a dietitian, a stress management instructor, and a licensed behavioral health specialist. Phase 2 provided additional reinforcement through monthly contact with a

health coach. The participants randomized to the control arm received no counseling but continued on with standard care provided by their primary care physicians.
Evaluation/Outcomes: Of the 145 participants, 58% were randomized to the intervention arm, 42% were men, and the mean age was 59 years. All participants underwent examinations at baseline, upon completion of the intervention, and 6 months later. At baseline, 60% of the patients had a known history of HTN. Although 96% of these patients were taking prescribed antihypertensive medication, 37% were clinically hypertensive (BP of 2:140/90) at the baseline examination. After completing the intervention, 75% of the participants were normotensive (BP of <140/90), with only 6% requiring an increase in medication. Through participation in the program, 12% of the patients were able to decrease or stop antihypertensive medication use. Importantly, changes in HTN status were maintained 6 months later. Conversely, no improvement was observed in 39% of the control participants who were clinically hypertensive at baseline.
Implications for Practice: A comprehensive program of lifestyle change that educates patients to make healthy lifestyle choices and provides ongoing access to health care professionals can successfully improve HTN in as little as 4 months in most patients, and these changes can be maintained at least 6 months later.

All aliquots for lipoprotein analysis (LipoScience) and plasma biomarkers (Johns Hopkins) were sent in 10 separate batches. A summary of all aliquots collected is shown below:

Aliquot/Biomarker	**# of Aliquots collected**
NMR Lipids	883
Leptin	883
CRP	883
Resistin	883
Insulin	883
Extra Plasma	2595
Adiponectin	890
Serum amyloid A	890
Vitamin D	890
Lp(a)	890
Extra Serum	3377
RBCs	1765
Paxgene	884
Serum clots	338
Total	16,934

Gene Expression – 97 U133A 2.0 expression arrays were completed on CRC samples (39 participants and 5 controls) with call rates ranging from 56.40-62.8% (average 59.95%); 228 CRC samples have been isolated (concentration range: 8.57- 326.50 ng/µl, average 91.52 ng/µl). Of the samples that have not been completed on arrays, 34 have been globin-cleared, amplified, and fragmented; an additional 57 CRC samples have been isolated and globin-cleared.

DMET arrays were run on DNA samples from 105 CRC participants; call rates were 98.65-100%, average 99.20%.

Task #6: Initiate "Exploring the Predictive Patterns of the Natural History of Pre-diabetes: Proof of Principle Study" protocol at WRNMMC (PI – COL Robert Vigersky, Diabetes Institute).

Status:
WRNMMC protocol submitted to DRP on 9 May 12 and IRB approval received on 6 Dec 12 with subsequent 2nd level approval by USAMRMC HRPO on 4 Jun 13. The WRI protocol was approved by the WMC IRB on 17 May 13 and by the USAMRMC HRPO on 24 July 2013. The WRI protocol was then submitted to the Chesapeake IRB on March 10, 2014, who determined this study was non-human subject research at WRI. A Sub award for Geneva Foundation was executed and study planning begun. Prior to the final execution of the Theranos, Inc. contract, a decision was made to close this study and reallocate funding to other projects.

Task #7: Continue study entitled "Metabolic and Biomolecular Biology Study Studies in Surgical Interventions for Morbid Obesity" as a component of the Integrative Cardiac Health Program at WRI.

Status:
USAMRMC approval received 15 June 12; protocol approved by Chesapeake IRB on 25 Feb 14. Continuing review of the protocol was approved by the Chesapeake IRB on 14 Jan 2015 (05-03). To date, the total enrollment is 280; 242 were still active participants, 38 drop-outs; 65 have only 1 follow-up, 45 have 2 follow-ups, and 59 have 3 or more.

Gene Expression – Below is a summary of gene expression arrays completed during the contract period:

Baseline	6Mo	1YR	18Mo	2YR	30Mo	3YR	42Mo	4YR	54Mo	5YR	66Mo	6YR	Total completed	
114	65	46	24	15	9	8	11	12	18	16	11	4	353	

Array call rates ranged from 54.4-61.2% (median 58.96%).

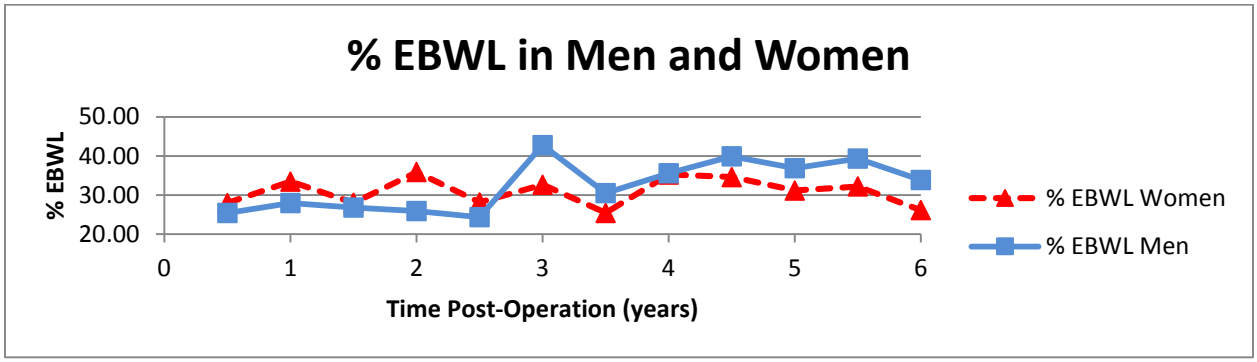
RNAs from 461 samples were isolated (concentration range 0-233.47 ng/µl, average 75.2 ng/µl). Of the samples that have not been completed on arrays, 29 have been globin cleared, amplified, and fragmented. There are an additional 4 Marley samples that have been isolated and globin cleared.

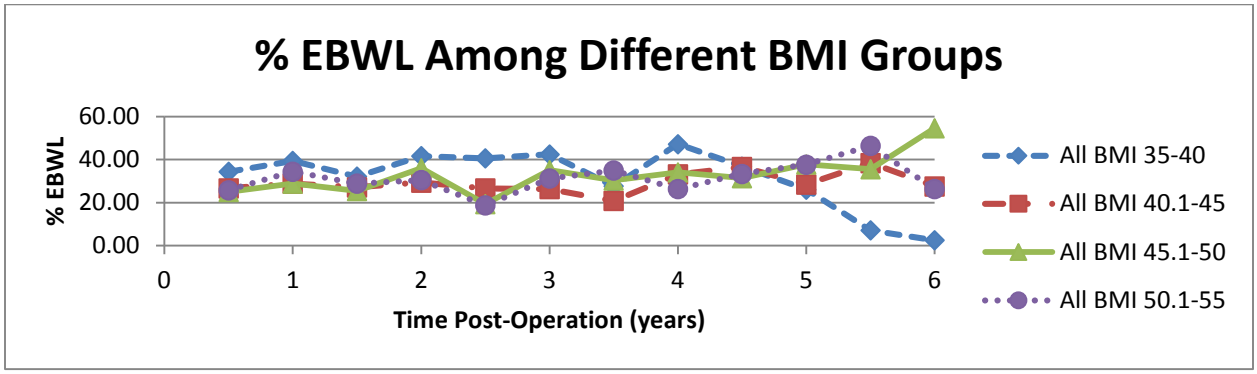
During the contract period, 9,852 plasma/RBC aliquots were processed and stored as outlined below:

Aliquot/Biomarker	# of Aliquots
NMR Lipids	605
Leptin	609
Insulin	608
CRP	609
Glucose	609
Low volume plasma	1803
RBCs	2234
Plasma	2775
Paxgene	637
Adipose (omentum & subcutaneous)	523
Total excluding Paxgene & adipose	9,852

All NMR lipids aliquots were sent to LipoScience and all other biomarkers were sent to Johns Hopkins in 4 separate shipments.

The percentage of excess body weight loss (%EBWL) was calculated. Trends in long-term weight loss were examined by random coefficients models and Kruskal-Wallis Rank-Sum Tests. Results show that there is no significant evidence (95% confidence level) that there are differences in %EBWL across time points for the group as a whole, other than that the overall group experiences positive %EBWL with respect to baseline. After 6 months, significant changes in %EBWL did not occur.





Comparison of lipoprotein changes in a surgical intervention vs lifestyle modification – The following abstract, which compared lipoprotein changes following a surgical intervention vs lifestyle modification, was presented as a Poster at the Obesity Society meeting.

Abstract Presented:

Blackburn HL, Mamula KA, Haberkorn MJ, Burke A, Slavik JE, Sann NJ, Marley KR, Vernalis MN, Ellsworth DL. Differential effectiveness of laparoscopically-adjustable gastric banding versus lifestyle modification for modifying plasma lipoprotein profiles. Obesity 2013: 31st Annual Scientific Meeting, Atlanta, GA, November 2013. (Poster)

Abstract

Obesity is an important cardiovascular disease (CVD) risk factor implicated in dyslipidemia and vascular dysfunction. Although LDL lowering is often a primary goal of therapy, the size and concentration of lipoproteins provide additional information on the true atherogenicity of plasma lipids. Surgical and lifestyle interventions are options for weight loss, but little is known about their effects on lipoproteins.

Changes in BMI and plasma lipoproteins over 1 year were compared between 31 patients undergoing laparoscopically placed adjustable gastric banding (LAGB) and matched participants in 2 lifestyle change programs differing in scope and intensity. Lipoprotein profiles were determined by nuclear magnetic resonance (NMR) spectroscopy. Baseline values were compared using Wilcoxon Signed Rank tests for matched pairs; changes over time were assessed by paired t-tests.

Over 1 year, LAGB led to significantly lower BMI (-16%, $p<0.001$ vs baseline) than intensive (-8%, $p<0.001$) or moderate (-2%, $p<0.05$) lifestyle change (matched-pairs $p<0.001$ in both comparisons). Notably, lipoprotein responses differed between interventions. Intensive lifestyle led to clinical changes in total LDL particles (–10%, $p<0.05$ vs baseline and matched pairs), while LAGB resulted in a significant increase in HDL particles (+19%, $p<0.001$) versus intensive (+1%) or moderate (–6%, $p<0.05$) lifestyle change (matched pairs $p<0.001$).

LAGB surgery and lifestyle change led to weight loss and changes in lipoprotein subclasses; however, the interventions may affect CVD risk through different pathways. Lifestyle reduced the atherogenicity of LDL lipoproteins, which may inhibit inflammation and endothelial dysfunction. Gastric surgery improved the number of HDL particles and may protect against CVD through anti-inflammatory and antioxidant activities.

Task #8: Initiate the "Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal: Long-term Follow-up Sub-study at WRI.

Study is assessing long-term maintenance (3-7+ years) of selected physical parameters, psychometric measures, plasma lipids, and peripheral blood gene expression in past participants of the Ornish Program to understanding whether traditional risk factor and molecular changes persist over time and contribute to long-term risk reduction.

Status:
Continuing review of the protocol was approved by the Chesapeake IRB on 14 Jan 2015 (12-03). Nine examinations were conducted for past participants; exams consisted of blood draw, psychometric surveys, standard anthropometric measurements, and 3-day dietary recall. Recruitment is complete; a total of 54 participants enrolled in the Ornish program and 39 controls participated in this study.

For all participants and controls, we collected and collated all data forms, completed all food diaries in Food Processor, scored psychosocial surveys, and entered all data including medication/vitamins and physical assessment forms into the database.

Blood was collected from 63 individuals originally in cohorts 14-25 or the control arm of the Ornish program. RNA was isolated from all 63 Paxgene tubes with concentrations ranging from 13.05-125.77 ng/µl, average 66.1 ng/µl). All aliquots for NMR lipids, insulin, leptin, and CRP were sent to LipoScience (NMR lipids) or Johns Hopkins for analysis. Expression arrays were completed on 61 of the samples (2 samples were excluded due to low RNA concentration) with expression call rates ranging from 58.60-63.12%, average 60.77%.

Blood was drawn for analysis from an additional 32 participants originally in cohorts 1-13. The total number of long-term follow-up aliquots collected is shown below:

Aliquots/Biomarker	**# of Aliquots**
NMR Lipids	63
CRP	63
Insulin	63
Leptin	63
Extra Plasma	416
RBCs	248
Paxgene	63
Total excluding Paxgene	916

The following Tasks were "terminated" after Year 1 of the award:

CORE Tasks

Task: Initiate "Lifestyle Education and Support Empowering prevention in breast disease patients (LEASE) Trial". Protocol development terminated due to an award re-budget.

CSI Tasks

Task: Continue "Stress Therapy Empowering Prevention (STEP) component to CRC. This program terminated due to low enrollment and a general lack of interest among women in the community.

This is a collaborative study involving researchers from WRI and WRNMMC and is modeled after the Caretakers Optimizing Readiness through Preventive Strategies (CORPS), designed by the ICHP at WRNMMC, except that it targets participants with chronic disease. The purpose of this task is to determine the degree of stress, sleep disturbance, and cardiovascular disease risk in patients who have been diagnosed with breast cancer or are at high risk of developing breast disease.

In the first part of the intervention, patients will be randomized to a 12 week Healthy Lifestyle intervention group or a non-intervention group. During this phase, each intervention participant undergo a comprehensive health risk assessment that is completed by a physician, followed by mandatory attendance to on-site group sessions in which they will participate in 1 hour of stress management, 30 minutes of nutrition education every week, and 30 minutes of exercise alternated with 30 minutes of mind/body health every other week. In addition, the nurse will provide educational lectures on various health topics during 4 sessions. After completing Phase I, patients will participate in a five year healthy lifestyle intervention or control group.

During phase II each intervention participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase II for five years, during which time they will come to the center for re-assessments every six months.

We hypothesize that the 12 week healthy lifestyle interventions will significantly reduce stress, sleep disturbances, and cardiovascular risk in patients at risk for, or already diagnosed with, breast cancer.

Status:
Study is closed. A manuscript describing the methods and preliminary results were published. The reference, abstract and key findings of the study are provided below.

Manuscript Published:

Burke A, Ellsworth DL, Vernalis MN. Stress Therapy Empowers Prevention (STEP): A Healthy-Lifestyle Program for Breast Cancer Patients. *J Oncol Navig Surviv* 2012;3(1):8-14.

Manuscript Abstract

Purpose: Develop and implement a comprehensive program for lifestyle change, empowering breast cancer patients to manage stress effectively and improve their mental and physical health.
Method: Women with breast disease (or those at high risk) are offered a program of lifestyle change, consisting of a Healthy Lifestyle intervention for 3 months followed by monthly contact with a health coach. Instruction and demonstration provide information on exercise, nutrition, stress reduction, and mind/body health. Examinations are conducted at baseline, after completion of the intervention (3 months), at 1 year, and every 6 months for a period of 5 years.
Conclusion: Breast cancer has a significant emotional, psychological, and social impact and is often associated with high levels of stress that promote unhealthy behaviors causing weight gain, decreased physical fitness, and an increased risk for cardiovascular disease (CVD). Similar to CVD, research shows breast cancer susceptibility is also influenced in part by modifiable risk factors, suggesting that a healthy lifestyle program may lead to reductions in cancer risk and recurrence as well as improvements in mental health and quality of life. Through the Stress Therapy Empowering Prevention (STEP) program, breast cancer and high risk patients are empowered with tools to focus on health promotion and optimization and maintenance of quality of life. Patients can improve physical and psychosocial factors in as little as 3 months, but long-term follow-up will determine if lifestyle changes result in improved clinical outcomes over time.

Key Findings:

Subject Enrollment and Demographics:

Total subject enrollment was 18 (intervention only); 10 active; 8 dropouts. Demographic characteristics of participants were: average age 65.6 years, 28% veterans or the spouse of a veteran, 6% have diagnosed coronary heart disease, and 61% have diagnosed breast cancer. Due to the lack of public interest we were unable to recruit a sufficient number of participants to keep this protocol open. The protocol was closed for enrollment on September 1, 2012 but will remain open for data analysis.

In the last quarter (July 2012- 15 Sept 2012) there were a total of 8 participant visits including periodic follow up phone calls made to participants.

Outcomes Data:

Overall participants showed change in the desired direction for most of the measured coronary artery disease (CAD) risk factors over the 2 years of the program (see Tables 11A-11D below). No participants were enrolled into the control arm of the study and lack of statistically significant levels of improvement in some measures may be attributable to small sample size and wide variability in some measures.

Table 11A. Comparison of baseline to Week 12 data for participants in the STEP Program

Category / Metrics	N	Average Baseline Value (SD)	Average Week 12 Value (SD)	Average Change	P Value
Weight (lbs.)	16	182.57 (35.9)	179.30 (33.0)	-3.3	<0.01
Body Mass Index	16	32.83 (6.3)	32.04 (5.9)	-0.8	<0.01

Total Cholesterol (mg/dl)	16	198.38 (36.4)	196.69 (44.2)	-1.7	0.7954
High Density Lipids (mg/dl)	16	54.44 (12.4)	52.25 (12.8)	-2.2	0.0928
Low Density Lipids (mg/dl)	16	114.50 (28.7)	118.63 (38.4)	4.1	0.5290
Triglycerides (mg/dl)	16	155.13 (90.6)	132.81 (73.4)	-22.3	0.0926
Systolic Blood Pressure	16	134.75 (18.8)	124.50 (14.1)	-10.3	0.0763
Diastolic Blood Pressure	16	80.63 (11.3)	73.75 (8.1)	-6.9	<0.05
Depression Scale [CES-D]	16	15.31 (10.2)	11.44 (10.4)	-3.9	0.0914
Hostility Scale [Cook-Medley]	16	7.06 (4.4)	5.25 (3.3)	-1.8	0.0720
Daily Total Fat (grams	8	58.62 (39.1)	44.48 (5.8)	-14.1	0.3394
Daily Saturated Fat (grams)	8	19.77 (19.5)	11.77 (3.4)	-8.0	0.2853
Perceived Stress Scale [PSS]	16	17.00 (7.2)	12.88 (6.5)	-4.1	<0.05
Avg. CCA/Mean IMT	16	0.735 (0.1488)	0.810 (0.1677)	0.075	<0.01
Avg. CCA / Max IMT	16	0.865 (0.1556)	0.928 (0.2046)	0.1	<0.05
Fasting Glucose (mg/dl)	16	107 (28.8)	109 (25.7)	2.4	0.6604
HgbA1c	16	6.3 (0.87)	6.5 (0.77)	0.2	0.3545
Cortisol	16	12.8 (3.83)	16.5 (5.44)	3.7	0.0507
TSH	16	1.71 (1.342)	2.07 (1.674)	0.4	0.2887
Epworth Sleepiness Scale (0 to 24)	16	9 (4.5)	8 (4.2)	-0.9	0.4320
Pittsburgh Sleep Quality Index (0-21)	16	10 (4.8)	8 (4.4)	-2.5	0.0512

Table 11B. Comparison of baseline to Year 1 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	14	180.49 (35.5)	177.30 (32.7)	-3.2	<0.05
Body Mass Index	14	32.49 (6.4)	31.66 (6.0)	-0.8	<0.05
Total Cholesterol (mg/dl)	14	201.07 (37.3)	200.21 (45.8)	-0.9	0.9083
High Density Lipids (mg/dl)	14	54.64 (13.1)	52.14 (13.7)	-2.5	0.0715
Low Density Lipids (mg/dl)	14	116.79 (30.0)	121.57 (40.2)	4.8	0.5252
Triglycerides (mg/dl)	14	157.21 (95.4)	136.71 (76.1)	-20.5	0.1721
Systolic Blood Pressure	14	134.00 (18.4)	125.14 (15.0)	-8.9	0.1451
Diastolic Blood Pressure	14	79.57 (11.3)	72.86 (8.3)	-6.7	0.0838
Depression Scale [CES-D]	14	13.71 (9.7)	11.29 (11.0)	-2.4	0.1056
Hostility Scale [Cook-Medley]	14	6.36 (4.2)	4.79 (3.2)	-1.6	0.3330
Perceived Stress Scale [PSS]	14	16.29 (7.4)	12.71 (6.8)	-3.6	<0.05
Daily Total Fat (grams)	10	61.64 (36.5)	54.64 (25.4)	-7.0	0.4039
Daily Saturated Fat (grams)	10	21.49 (18.2)	15.26 (8.5)	-6.2	0.1507
Avg. CCA/Mean IMT	14	0.745 (0.1557)	0.826 (0.1718)	0.081	<0.01
Avg. CCA / Max IMT	14	0.879 (0.1607)	0.948 (0.2110)	0.1	<0.05
Fasting Glucose (mg/dl)	14	109 (30.6)	111 (27.4)	2.0	0.7535

HgbA1c	14	6.4 (0.91)	6.6 (0.77)	0.2	0.3356
Cortisol	14	12.4 (3.61)	16.9 (5.61)	4.5	<0.05
TSH	14	1.66 (1.419)	2.19 (1.766)	0.5	0.1559
Epworth Sleepiness Scale (0 to 24)	14	8 (4.5)	8 (4.5)	-0.6	0.6020
Pittsburgh Sleep Quality Index (0-21)	14	10 (5.1)	8 (4.6)	-2.7	0.0639

Table 11C. Comparison of baseline to 18 month data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	10	189.23 (36.5)	188.36 (33.3)	-0.9	0.7195
Body Mass Index	10	33.66 (7.0)	33.37 (6.2)	-0.3	0.4956
Total Cholesterol (mg/dl)	10	200.50 (40.4)	211.50 (60.3)	11.0	0.4066
High Density Lipids (mg/dl)	10	53.50 (13.3)	52.00 (13.2)	-1.5	0.3974
Low Density Lipids (mg/dl)	10	113.40 (31.1)	126.60 (43.3)	13.2	0.1605
Triglycerides (mg/dl)	10	180.80 (103.6)	163.20 (115.1)	-17.6	0.3961
Systolic Blood Pressure	10	135.80 (20.2)	137.40 (18.0)	1.6	0.8362
Diastolic Blood Pressure	10	79.40 (12.6)	75.20 (11.8)	-4.2	0.2921
Depression Scale [CES-D]	10	13.20 (9.6)	6.80 (7.1)	-6.4	<0.05
Hostility Scale [Cook-Medley]	10	7.40 (4.4)	5.90 (3.6)	-1.5	0.1604
Perceived Stress Scale [PSS]	10	16.00 (8.2)	8.90 (6.8)	-7.1	<0.01
Daily Total Fat (grams)	10	61.64 (36.5)	37.96 (12.0)	-23.7	0.0766
Daily Saturated Fat (grams)	10	21.49 (18.2)	10.20 (4.2)	-11.3	0.0943
Avg. CCA/Mean IMT	10	0.751 (0.1695)	0.775 (0.1719)	0.024	0.4697
Avg. CCA / Max IMT	10	0.888 (0.1815)	0.919 (0.2275)	0.0	0.4407
Fasting Glucose (mg/dl)	10	110 (34.8)	112 (39.7)	1.8	0.6648
HgbA1c	10	6.4 (0.98)	6.7 (1.52)	0.3	0.3126
Cortisol	10	12.8 (3.83)	13.1 (4.67)	0.3	0.8983
TSH	10	1.64 (1.577)	1.44 (1.243)	-0.2	0.5141
Epworth Sleepiness Scale (0 to 24)	10	8 (4.7)	7 (4.0)	-0.7	0.5496
Pittsburgh Sleep Quality Index (0-21)	10	10 (5.0)	7 (3.4)	-3.1	0.1121

Table 11D. Comparison of baseline to year 2 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	10	189.23 (36.5)	184.84 (31.3)	-4.4	0.1403
Body Mass Index	10	33.66 (7.0)	32.58 (5.7)	-1.1	0.1971
Total Cholesterol (mg/dl)	10	200.50 (40.4)	194.60 (53.8)	-5.9	0.5141

High Density Lipids (mg/dl)	10	53.50 (13.3)	48.70 (11.9)	-4.8	<0.001
Low Density Lipids (mg/dl)	10	113.40 (31.1)	114.70 (43.3)	1.3	0.8637
Triglycerides (mg/dl)	10	180.80 (103.6)	162.20 (118.8)	-18.6	0.4207
Systolic Blood Pressure	10	135.80 (20.2)	132.60 (15.3)	-3.2	0.4981
Diastolic Blood Pressure	10	79.40 (12.6)	76.40 (10.6)	-3.0	0.3412
Depression Scale [CES-D]	10	13.20 (9.6)	12.00 (11.9)	-1.2	0.7045
Hostility Scale [Cook-Medley]	10	7.40 (4.4)	6.40 (5.3)	-1.0	0.3765
Perceived Stress Scale [PSS]	10	16.00 (8.2)	13.00 (9.1)	-3.0	0.2401
Daily Total Fat (grams)	10	61.64 (36.5)	56.12 (20.1)	-5.5	0.7037
Daily Saturated Fat (grams)	10	21.49 (18.2)	17.05 (6.0)	-4.4	0.5093
Avg. CCA/Mean IMT	10	0.751 (0.1695)	0.738 (0.1484)	-0.013	0.7605
Avg. CCA / Max IMT	10	0.888 (0.1815)	0.845 (0.1683)	0.0	0.3379
Fasting Glucose (mg/dl)	10	110 (34.8)	111 (30.9)	0.6	0.8474
HgbA1c	10	6.4 (0.98)	6.4 (0.94)	0.0	0.5987
Cortisol	10	12.8 (3.83)	14.7 (4.31)	1.8	0.1363
TSH	10	1.64 (1.577)	2.22 (1.635)	0.6	0.2716
Epworth Sleepiness Scale (0 to 24)	10	8 (4.7)	7 (4.5)	-0.7	0.6380
Pittsburgh Sleep Quality Index (0-21)	10	10 (5.0)	7 (4.3)	-3.1	0.0895

Adverse Events:
All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. To date, there have been 5 adverse events, 4 were deemed serious and 1 event was not serious. Three of the events were considered serious due to inpatient hospitalizations and one due to poor prognosis related disease progression. No deaths occurred and none of these adverse events were deemed to be study related.

Task: Continue "Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women" at WRI and initiate "Isolation, Amplification, and Genotyping of DNA from Serum Samples in the Department of Defense Serum Repository (DODSR): A Proof of Principle Study" protocol at WRNMMC/WRI.

Methodology:
The purpose of this proof-of-concept, feasibility, laboratory-based study, utilizing serum samples obtained from the DoDSR was to: 1) assess the quantity and quality of DNA isolated from serum samples obtained from the DoDSR, 2) conduct whole-genome amplification of the serum DNA and evaluate the resulting whole-genome amplified DNA (wgaDNA), and 3) determine the feasibility of using the DoDSR wgaDNA on high-density genetic marker arrays for future studies. Fifty (50) orphan serum samples from individual service members that have been stored for different lengths of time and meeting specific inclusion/exclusion criteria would be obtained from the DoDSR and anonymized at the source. This study proposed to utilize two innovative technologies – whole-genome DNA amplification from stored serum specimens and whole genome characterization using advanced microarray technology – to determine the feasibility of using serum samples stored under the conditions of the DMSS/DoD Serum Repository for genome wide association studies. Studies also included collaboration with Vanderbilt University.

Status:
The initial WRI study was approved in 2008. The WRNMMC and WRI Proof-of-Principal studies received initial approvals in March 2012 and 2nd level approval in July 2012 with a "No Human Use" determination. Approval was also received from Vanderbilt University IRB. Initial discussions begun with the DoDSR for selection and release of samples, but after no response from DoDSR to begin further discussions, a determination was made to terminate this work. Closure documents for the WRNMMC study was submitted in April 2013 with subsequent WRNMMC DRP acknowledgement in September 2013; all documents were forwarded to USAMRMC. The WRI studies were closed in October 2013 and documents forwarded.

Initial research and development work was performed at WRI. Using laboratory samples that should be similar to the repository samples, call rates for all genomic DNA samples were all >97.90% (Table 12). Call rates for DNA isolated from serum were >93.00% and for DNA isolated from heparin plasma were >95.7%. Samples from EDTA tubes that were whole-genome amplified did not perform well (~69-89% call rates). Plans were to compare these serum samples with the samples from the DoDSR.

Table 12. Call rates on Affymetrix 6.0 arrays for DNA from various sources.

Sample	P/S	CQC	Call Rate
#1 Genomic	N/A	3.04	98.78
#2 Genomic	N/A	2.63	97.9484
#3 Genomic	N/A	2.43	97.9153
#5 Genomic	N/A	2.67	98.0477
#1 Serum Unamplified	Serum	0.72	93.051
#2 Serum Unamplified	Serum	2.32	97.7498
#3 Serum Unamplified	Serum	2.21	98.2793
#5 Serum Unamplified	Serum	2.25	97.85
#1 Serum WGA	Serum	2.19	96.1946
#2 Serum WGA	Serum	-0.05	84.71
#3 Serum WGA	Serum	2.15	96.1284
#5 Serum WGA	Serum	0.72	90.7346
#1 EDTA Unamplified	Plasma	0.47	89.74
#2 EDTA Unamplified	Plasma	3.31	99.01
#3 EDTA Unamplified	Plasma	-0.46	87.7895
#5 EDTA Unamplified	Plasma	0.43	91.4295
#1 EDTA WGA	Plasma	-0.07	77.9616
#2 EDTA WGA	Plasma	0.01	88.88
#3 EDTA WGA	Plasma	-0.03	68.63
#5 EDTA WGA	Plasma	-0.06	73.0311
#1 Heparin Unamplified	Plasma	1.67	95.7313
#2 Heparin Unamplified	Plasma	2.6	97.1873
#3 Heparin Unamplified	Plasma	2.6	98.84
#5 Heparin Unamplified	Plasma	2.41	98.1469
#1 Heparin WGA	Plasma	2.02	94.143
#2 Heparin WGA	Plasma	2.75	98.5109
#3 Heparin WGA	Plasma	2.21	97.3527
#5 Heparin WGA	Plasma	2.61	97.9815

Based on this initial work, the following abstracts with references are listed below.

Abstract Presented:

Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. *J Mol Diag* 2011;13(6):780.

Presented at Association for Molecular Pathology (AMP) 2011 Annual Meeting, Grapevine, TX, November 2011. (Poster)

Abstract

Introduction: The ability to obtain sufficient high quality DNA from archival formalin fixed, paraffin-embedded (FFPE) tissue often limits genomic analysis for researchers and clinicians alike. Of numerous methods developed to optimize the quantity of DNA extracted from FFPE tissues, whole genome amplification (WGA) has become a robust and reliable technique for obtaining sufficient genomic material for a variety of molecular applications. Previous studies suggest that DNA obtained from FFPE samples may be used on high-density single nucleotide polymorphism (SNP) arrays to provide information on SNP genotypes, chromosome copy number (CN), and loss of heterozygosity, but spurious results occur with insufficient DNA template.

Methods: We examined the feasibility of assessing chromosome CN variation using whole genome amplification on DNA extracted from FFPE tissue, as well as fresh frozen (FF) tissue in OCT, and high-density Affymetrix GeneChip® 500K SNP Mapping Arrays. Genomic DNA was extracted from microdissected regions (approx 2.9 mm^2) of human tissue preserved in paraffin using the GenomePlex® Tissue Whole Genome Amplification Kit (Sigma®) and from human FF tissue using QiaAmp® DNA Mini Kit (Qiagen®). Whole-genome amplification was then performed on 1.5 µI of FF or FFPE DNA using the REPLI-g® whole-genome amplification kit (Qiagen®). Genotypes were determined using the Dynamic Model Mapping Algorithm in the Affymetrix GeneChip® Genotyping Analysis Software (GTYPE 4.0) package and CN variation was assessed with Genotyping Console™ (Affymetrix).

Results: Acceptable genotyping call rates were obtained for all unamplified DNA samples (96.3 ± 1.5%) and wgaDNA samples (93.3 ± 1.6%) from FF tissue. Call rates were significantly lower however, for wgaDNA samples from FFPE (67.5 ± 5.1%) ($P<0.001$). Assessment of CN variation was highly consistent between unamplified and whole-genome amplified FF samples, but was clearly discordant between amplified FF and amplified FFPE samples.

Conclusions: These results indicate that FF tissue, even if whole-genome amplified, is useful for genome-wide SNP genotyping and determining chromosome CN variation, but large discrepancies are likely to occur when using whole-genome amplification on DNA isolated from FFPE. CN variation may be affected by uneven amplification of the genome with small quantities of suboptimal DNA template extracted from FFPE samples.

Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, Grapevine, TX, November 2011. (Poster)

Abstract

Introduction: Defining genetic variation associated with complex human diseases requires high-quality DNA from well-characterized patients. With the development of robust technologies for whole-genome amplification, sample repositories such as serum banks now represent a potentially valuable source of DNA for genomic studies and clinical diagnostics. We assessed the performance of whole-genome amplified (wga) DNA derived from stored serum/plasma for estimating chromosome copy number (CN) variation on high-density single nucleotide polymorphism (SNP) arrays.

Methods: Fresh serum and plasma samples were obtained from subjects who voluntarily agreed to participate in this study and gave written informed consent. DNA was extracted from 200 µL

of serum or plasma using the QIAamp® DNA Blood Mini Kit Genomic (g) DNA was isolated from peripheral blood mononuclear cells with the Puregene® DNA Purification Kit according to the manufacturer's protocol. Whole-genome amplification was then performed on 2.5 µL of serum/plasma DNA using the REPLI-g® whole-genome amplification kit. Genotypes were determined using Affymetrix GeneChip® Genotyping Analysis Software and CN variation was assessed with Genotyping Console™.
Results: Storage time and usage history did not affect DNA extraction or whole-genome amplification yields; however, samples that had been thawed and refrozen showed significantly lower call rates (73.9 + 7.8%) compared to samples that had never been thawed (92.0 + 3.3%) ($P<0.001$). Genotype call rates did not differ significantly ($P=0.13$) between wgaDNA from never-thawed serum/plasma (92.9 + 2.6%) and gDNA (97.5 + 0.3%) isolated from whole blood. Approximately 400,000+ genotypes were consistent between wgaDNA and gDNA; however, patterns of CN variation were highly discordant between serum/plasma wgaDNA and gDNA from the same patients. The CNV in the wgaDNA samples showed spurious regions of amplifications and deletions compared to the unamplified gDNA. These regions showed much larger areas of amplification and deletions across all the chromosomes compared to the unamplified gDNA CNV.
Conclusions: While use of stringent quality control requirements can facilitate the collection of quality SNP genotype data from wgaDNA, our data suggest that more advanced analyses, such as CN and loss of heterozygosity assessments, may be compromised due to spurious amplification during the whole- genome amplification process.

Task: Initiate "Young Service Members with Myocardial Infarction" protocol.

Status:
Without the results of the above 2 studies, we could not initiate this protocol, therefore, task was terminated.

Task: Initiate "Exploring the Predictive Patterns of the Natural History of Pre-diabetes: Proof of Principle Study" protocol (ICHP-WRNMMC in collaboration with WRNMMC Diabetes Institute and WRI).

Methodology:
The primary purpose of this prospective, observational, proof of principle study was to determine the feasibility of using a novel, point-of-care (i.e. home), multiple analyte test platform (Theranos) to study the temporal changes in five biomarkers related to glucose dysregulation, inflammation, vascular dysfunction, and immunity that can lead to diabetes and increased cardiovascular risk [insulin, leptin, high sensitivity Troponin T (hs-cTnT), high sensitivity C-reactive protein (hs-CRP), and ferritin]. A secondary purpose was to examine patterns of gene expression in peripheral blood in patients diagnosed with pre-diabetes who are entering into an intensive lifestyle modification program.
Up to 50 adult military healthcare beneficiaries ($\geq$ 18 years) who met the screening criteria for pre-diabetes and have self-referred or been referred to the ICHP-CPP for CV risk reduction were to be enrolled. Each participant would be provided a portable, home-based Theranos system and be asked to provide a fingerstick (FS) blood sample to the system at three specific times per week for 2 months pre-initiation and for the duration of their participation in the lifestyle change program.

Blood samples would be collected prior to (2 months) and at the conclusion of the lifestyle program (8 months) to evaluate changes in gene expression and to determine changes in the biomarkers noted above. Blood samples were to be collected again at 12, 24, and 36 months to determine if there were additional changes in the genetic markers and if the biomarkers were a measure of their dysglycemia.

A variety of statistical techniques were to be used, depending on the level of measurement of the variables being modeled (e.g., binomial, multinomial, continuous) to characterize the dynamic relationship between the analytes obtained by the Theranos system and 1) metabolic and CV risk and 2) advancement to diabetes and/or CVD.

Status:
WRNMMC protocol submitted to DRP on 9 May 12 and IRB approval received on 6 Dec 12 with subsequent 2nd level approval by USAMRMC HRPO on 4 Jun 13. The WRI protocol was approved by the WMC IRB on 17 May 13 and by the USAMRMC HRPO on 24 July 2013. The WRI protocol was then submitted to the Chesapeake IRB on March 10, 2014, who determined that this study was non-human subject research at WRI. Sub award for Geneva Foundation executed and study planning began. Annual continuing review was approved 14 Nov 2014, but protocol closed as of 5 Feb 2015 due to re-programming of allocated funds. Study planning had commenced, but no recruitment of subjects took place. MRMC HRPO Withdrawal Acknowledgement received Jun 2015.

KEY RESEARCH ACCOMPLISHMENTS

- **ICHP's innovative technology promotes patient engagement and adherence to behavior change with the Personalized Healthy eLifestyle Prescriptions.** ICHP's protocols are managed by the Research Information Management System (RIMS) which houses a web-based CV health assessment survey mechanism and Patient-provider web-portal for patient connectivity. Most importantly, ICHP is equipped to examine patient safety data for best clinical practices by utilizing automated audits.

- **Scientific research findings dissemination continues:**
  - 10 manuscripts published; 1 in press
  - 19 abstracts published in peer review journals
  - 29 abstracts presented at international and local scientific sessions
    - 22 posters presentations (1 moderated session)
    - 7 podium presentations
    - Poster presented at PCNA (2015) selected for moderated session; received 2nd place ribbon in research competition
    - Poster presented at ACC 2015 Scientific Conference: "Best CV Team" award
    - Poster presented at PCNA (2013): 1st place winner-Innovation in Patient Care Category
  - 2 abstracts accepted for poster presentations

Below are highlighted some of our key research findings:

- A pioneer in CV health, ICHP was first to add (2006) both stress and sleep improvement as **critical outcome measures to its model of CV health**.[8] These parameters are now widely recognized by the national scientific community but not yet added to the AHA's Strategic Impact Goals of 2020.

- To translate the evidence of the sleep and stress connection into practice, ICHP created a novel yet simple, **portable 10-minute stress reduction technique**, "Tension Tamer", to relieve stress, improve sleep quality and decrease fatigue. Of 334 patients using this technique, 65% improved their perceived stress by 6.6 points, while those not improving showed worsened stress levels by 4.6 points. Improvers also reported better sleep quality, decreased sleep latency, and decreased fatigue. This study finding was presented during CHEST 2012, the annual meeting of the American College of Chest Physicians and also received national press coverage.[9]

- We are the **first to show that gene expression is significantly modulated by sustained lifestyle behavior change**, which has beneficial effects on the vascular system not apparent from traditional risk factors. Healthy lifestyle behaviors also appear to restore balance in bodies where dysregulation of hormones cause the first stages of CV disease. In addition, we identified that lifestyle modification affects CVD risk in women through different mechanisms than in men.[10]

- ICHP **impacted patient safety with Clinical Guidelines on a national level by being cited as primary evidence** for the incorporation of Family History (FH) of premature CVD in comprehensive risk assessment by the AHA and ACC Expert Panel 2013 and enhancing CVD risk assessment.[4] The incorporation of FH into risk stratification: 1) Enhances CVD risk assessment by identifying previously unrecognized high-risk patients; 2) Reduces variability in practice, and; 3) Targets appropriate (more stringent) therapeutic goals for prevention.

- In *Circulation Cardiovascular Genetics*, successful and sustained modulation of gene expression through lifestyle changes may have **beneficial effects on the vascular system not apparent from traditional risk factors**. Healthy lifestyles may restore homeostasis to the leukocyte transcriptome by down-regulating lactoferrin and other genes important in the pathogenesis of atherosclerosis.[11]

- Our research findings published in *Sleep and Breathing* (2015)[12] and *CHEST* (2014)[13] suggest that healthcare p**roviders can better capture <u>Obstructive Sleep Apnea</u> in women by using the proper questionnaire tool to screen for fatigue** and not rely solely on assessments of sleepiness. Future clinical guidelines should incorporate this recommendation to avoid under-recognition of sleep pathology in women.

- Other ICHP genomic publications in *Genomics Data* suggest successful and **sustained modulation of gene expression through healthy lifestyle changes may have beneficial effects on vascular health** that cannot be discerned from traditional risk factor profiles. The data are deposited in the Gene Expression Omnibus, series GSE46097 and GSE66175.[14]

- In 2015, published in Obesity, ICHP was the first to demonstrate **that substantial weight loss (>10%) during lifestyle modification for improved CV health is associated with down regulation of genetic pathways governing interactions between circulating immune cells and vascular endothelium**.[15]

- In translating the evidence, ICHP created a new, no-cost clinical-decision support tool (CDST) to better identify CVD risk. This tool incorporates FH history of premature CVD augmenting the widely used FRS and **improving classification of a patient's true risk by 48%**. This CDST not only allows for patients to detect disease at an earlier stage but also has proven to help increase self-efficacy in patients with family history of premature heart disease.[16,17]

- ICHP received a great deal of press coverage for its publication in the *Journal of the American College of Cardiology* highlighting its unique approach to LMIs and preclinical disease.[18] Of the 107 participants who had prediabetes at the start of the ICHP-CHP study, 49% were at normal blood glucose levels at the end of the 6-month study period irrespective of weight loss. Combating progression to diabetes **with ICHP's practical LMI** reduces CVD risk and improves overall health in this vulnerable population.

- ICHP-CHP findings in agree with prior reports that high self-efficacy correlates with healthful diet and exercise habits. We extend this association to include better sleep quality

and less fatigue. These findings suggest that **efforts to increase self-efficacy benefit both traditional measures of CV health as well as encompass non-traditional measures, such as sleep health**.[19]

- ICHP was able to demonstrate that men and women present with CVD risk differently in the *Journal of Cardiovascular Nursing*. Traditionally, CVD risk scores rate women lower than men despite women's worse lipid profiles and the presence of non-traditional precursors for CVD risk such as higher rates of depression/anxiety and perceived stress, all of which may manifest overt disease after menopause. **ICHP-CHP LMIs show that in order to target interventions appropriately, screening approaches for CVD risk should aim to capture sex-specific vulnerabilities for CVD**.[20]

- The ICHP-CHP LMI published in *Journal for Global Health*, emphasizing **empowerment strategies early in the sequence of the program, improves self-efficacy leading to substantial behavioral improvements in CV health** parameters (diet, exercise, stress, sleep). These findings are highly relevant particularly in high-risk individuals who are vulnerable to CV disease.[21,22]

- ICHP's clinical algorithms have proven successful because they employ a standardized **Multidisciplinary Clinical Team Evaluation Process** combined with Customized Motivational Coach systems for sustainment. Out of 463 patients, ICHP's review evaluation **identified a significant number of pre-clinical states**: 19% pre-hypertension, 48% sleep apnea, 64% overweight and 31% pre-diabetes. Working as an adjunct to best medical practice, ICHP reported all new pre-clinical cases back to the patient's primary care provider.

REPORTABLE OUTCOMES

MANUSCRIPTS:

Ellsworth DL, Costantino NS, Blackburn HL, Engler, RJM, Kashani M, Vernalis MN. Lifestyle modification interventions differing in intensity and dietary stringency improve insulin resistance through changes in lipoprotein profiles. *Obes Sci Pra* 2016 (In press).

Kashani M, Eliasson AH, Walizer EM, Fuller CE, Engler RJ, Villines TC, Vernalis MN. Early empowerment strategies boost self-efficacy to improve cardiovascular health behaviors. *Glob J Health Sci* 2016 Feb 2;8(9):55119. doi: 10.5539/ghis.v8n9p322.

Kashani M, Eliasson A, Vernalis M, Bailey K, Tehaar M. A systematic approach incorporating family history improves identification of cardiovascular disease risk. *J of Cardiovasc Nurs* 2015 Jul-Aug;30(4):292-297. doi: 10.1097/JCN.0000000000000163. Epub 2014 May 20.

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Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Targeted behavioral interventions improve disturbed sleep. *SLEEP* 2016; 39:A397.

Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. *Ann Behav Med* 2016 Mar;50 Suppl 1:S124. doi: 10.1007/s12160-015-9766-4

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Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. *Circulation* 2012;126:A14216.

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PRESENTATIONS:

Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. targeted behavioral interventions improve disturbed sleep. APSS, Denver, CO, June 2016. (Poster)

Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. Society of Behavior Medicine (SBM) 37th Annual Meeting & Scientific Session, Washington DC, March 2016. (Poster)

Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Vernalis MN. Cardiac interventions differing in lifestyle modification improve insulin resistance through changes in lipoprotein profiles. American Heart Association (AHA) EPI/Lifestyle 2016 Scientific Sessions, Phoenix, AZ, March 2016. (Poster)

Kashani M, Eliasson A, Walizer E, Fuller C, Engler R, Villines T, Vernalis M. Early empowerment strategies boost self-efficacy to improve health outcomes. American Heart Association (AHA) 2015 Scientific Session, Orlando, FL, November 2015. (Poster)

Vernalis MN, Engler RJM, Mamula KA, Blackburn HL, Kashani M, Ellsworth DL. Weight loss impact on insulin resistance: A novel lipoprotein insulin resistance index (LP-IR) identifies differing phenotypes of response to lifestyle intervention. Military Health System Research Symposium (MHSRS), Fort Lauderdale, FL, August 2015. (Podium)

Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. High self-efficacy may benefit sleep quality and fatigue. Associated Professional Sleep Societies (APSS), Seattle, WA, June 2015. (Poster).

Kashani M, Eliasson A, Engler R, Villines T, Vernalis M. Women present with non-traditional precursors of CVD. Preventive Cardiovascular Nurses' Association 21st Annual Symposium (PCNA), Anaheim, CA, April 2015. (Poster/Moderated Session - received 2nd place ribbon in research competition)

Ellsworth DL, Mamula KA, Blackburn HL, Engler RJM, and Vernalis MN. Cardiac lifestyle interventions differing in dietary stringency improve insulin resistance through changes in lipoprotein profiles. The American College of Cardiology (ACC) 64th Annual Scientific Session, San Diego, CA, March 2015. (Poster)

Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Fuller C, Villines T, Vernalis M. Prediabetes reversal using a novel comprehensive health model. The American College of Cardiology 64th Annual Scientific Session, San Diego, CA, March 2015. (Poster – received recognition as "Best CV Team" award)

Eliasson AH, Kashani MD, Doody MM, Jones MK, Vernalis MN. Fatigue in women is a key symptom in evaluation of sleep apnea. CHEST 2014, Austin, TX, October 2014. (Poster)

Walizer EM, Vernalis MN, Modlin RE. Influence of CIMT as a motivator for health behavior change in a heart health program. American Heart Association (AHA) EPI/NPAM 2014 Scientific Session, San Francisco, CA, March 2014. (Poster)

Vernalis, MV. The results of studies of cardiovascular disease risk and prevention in the military population. Osteopathic Medical Conference & Exposition: 57th Annual Research Conference, Las Vegas, NV, September 2013. (Podium)

Blackburn HL, Mamula KA, Haberkorn MJ, Burke A, Slavik JE, Sann NJ, Marley KR, Vernalis MN, Ellsworth DL. Differential effectiveness of laparoscopically-adjustable gastric banding versus lifestyle modification for modifying plasma lipoprotein profiles. Obesity 2013: 31st Annual Scientific Meeting, Atlanta, GA, November 2013. (Poster)

Eliasson AH, Kashani M, Bailey K, Vernalis M. Sleep quality improves in adherents to heart health program without change in sleep duration. Affiliated Professional Sleep Society Meeting, Baltimore, MD, June 2013. (Poster)

Bailey K, Kashani M, Eliasson A, Vernalis M. Low self-efficacy correlates with increased cardiovascular disease risk. AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2013 Scientific Session, Baltimore, MD, May 2013. (Poster)

Kashani M, Eliasson A, Bailey K, Vernalis M. Systematic inquiry of family history improves CV risk assessment. AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2013 Scientific Session, Baltimore, MD, May 2013. (Poster)

Walizer EM, Vernalis MN, Modlin RE. Adherence to a lifestyle intervention program not improved by visual knowledge of carotid intima atherosclerosis. AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2013 Scientific Session, Baltimore, MD, May 2013. (Poster)

Burke A, Ellsworth DL, Haberkorn MJ, Lechak F, Sullivan J, Adams B, Patney HL, Mamula KA, Vernalis MN, Kashani M. Coaching patients to control hypertension through a team-based, patient-centered program: the cardiovascular risk clinic. Preventive Cardiovascular Nurses Association: PCNA 19th Annual Symposium, Las Vegas, NV, May 2013. (Poster-1st place winner: Innovation in Patient Care category)

Bittman B, Ellsworth DL, Vernalis MN. Stress reduction through creative musical expression impacts biological pathways on the DNA level in individuals with coronary heart disease. National Summit: Arts, Health & Well-being Across the Military Continuum, National Initiative for Arts & Health in the Military, National Endowment for the Arts, Walter Reed National Military Medical Center, Bethesda, MD, April 2013. (Podium)

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. American Society of Human Genetics, San Francisco, CA, November 2012. (Poster)

Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. American Heart Association Scientific Sessions 2012, Los Angeles, CA, November 2012. (Poster)

Eliasson A, Kashani M, Vernalis M. Sleepy on Venus, Fatigued on Mars? TriService American College of Physicians, Bethesda, MD, November 2012. (Podium)

Modlin RE, Walizer EM, Vernalis MN. CIMT imaging knowledge effect on lifestyle program adherence. TriService American College of Physicians, Bethesda, MD, November 2012. (Podium)

Eliasson AH. Sleep—the Year in Review. TriService American College of Physicians, Bethesda, MD, November 2012. (Podium)

Kashani M, Eliasson A, Bailey K, Vernalis M. Novel stress reduction technique improves sleep and fatigue. American College of Chest Physicians, Atlanta, GA, October 2012. (Podium)

Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. The importance of weight loss for effecting molecular change during intensive cardiovascular risk reduction. Obesity 2012: 30th Annual Scientific Meeting, San Antonio, TX, September 2012. (Poster)

Eliasson A, Kashani M, Vernalis M. Sleepy on Venus, Fatigued on Mars? American Thoracic Society, San Francisco, CA, May 2012. (Poster)

Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, Grapevine, TX, November 2011. (Poster)

Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, Grapevine, TX, November 2011. (Poster)

DEGREES SUPPORTED BY THIS AWARD:

Mariam Kashani, Chief Scientific Officer, Completion date: May 2013
Doctorate of Nursing Practice (DNP) – Johns Hopkins University, Baltimore, MD

Lydia Hill, Program Manager I, Completion date: Unknown
Bachelors of Science, Healthcare Administration

INFORMATICS:

ICHP contracted with MDM Technologies, through the Henry M. Jackson Foundation, to implement a web-based Clinical Information Management System to support program operations and to obtain, update, store and report on participant data collected by all members of the ICHP clinical team and to support its patient management, diagnostic testing, clinical monitoring and clinical research for all protocols.

The scope of this project was defined in 9 high-level functional areas as follows:

1. Survey packet creation with scoring (web-based capability & calculation of derived)
2. Data input module creation (with field parameters for QA&QC) (audit trail & queries)
3. Data output module creation -- clinical review summary view
4. Data output module creation -- individual patient and provider reports (email also)
5. Data output module creation-- automated and customized aggregate reports over time
6. Mechanism to ID participants (also blind, restrict access and re-id)
7. CRF creation and management (coding and tracking)
8. Data migration
9. Customized scheduling and visit module for program and protocols (trigger events, input modules per appointment type, track workload productivity)

CONCLUSION

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could substantial reduce the development of CHD. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. We also have shown that molecular change occurs during lifestyle modification, but this change may be transient and may be dependent on maintaining a healthy lifestyle. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

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APPENDIX A

LIST OF PERSONNEL RECEIVING PAY FROM RESEARCH EFFORT

WRNMMC PERSONNEL	
Bailey, Karla	Data Outcomes Specialist
Caporiccio, Christa	Administrative Assistant
Crosniak, Linda	Clinical Psychology Consultant
Doody, Meghan	Nurse Practitioner
Edinger, Rosemarie	Chief Nursing Officer
Eliasson, Arn	Senior Physician Research Consultant
Engler, Renata	Senior Research Physician Consultant
Fuller, Claire	Clinical/Administrative Office Manager
Grunewald, Marilyn	Stress Reduction Specialist
Halsey, Joy	Clinical Dietitian
Henderson, Josephine	Administrative Assistant
Hill, Lydia	Program Manager
Hoffman, Jacqueline	Stress Reduction Specialist
Hunt, LaBelle	Administrative Assistant
Jones, Marian	Nurse Practitioner
Kashani, Mariam	Chief Scientific Officer
Lalicato, Amanda	Nurse Practitioner
Lampkin, Bettina	Senior Financial Analyst
Llewellyn, Peta-Gay	Sonographer
Matthews, Nansy	Nurse Practitioner
Nixon, Audra	Director, Administration
Reid, Gloria	Nurse Practitioner
Turner, Ellen	Exercise Physiologist/Health Coach
Tschiltz, Nancy	Clinical Dietitian
Vernalis, Marina	Principal Investigator/Medical Director
Walizer, Elaine	Director, Clinical Research Coordination
Williams, Kenneth	Senior Financial Analyst
WINDBER RESEARCH INSTITUTE PERSONNEL (Subaward)	
Blackburn, (Patney) Heather	Research Associate III
Blankenship, Sara	Research Associate II
Chen, Yaqin	Intern
Costantino, Nick	Sr. Statistical Analyst
Croft, Dan	Research Associate III
Decewicz, Alisha	Research Associate II
Decewicz, David	Research Physician
Ditton, Dana	Research Assistant

Ellsworth, Darrell	Senior Director/Chief Clinical Apps. Officer
Elston, Ed	IT Manager
Furmanchik, Lydia	Finance Assistant
Greenawalt, Amber	Research Assistant
Kohr, Joni	Administrative Assistant II
Latoche, Joseph	Research Associate II
Mamula, Kim	Sr. Statistical Analyst
Melley, Jen	Research Associate II
Mural, Richard	Chief Scientific Officer
O'Donnell, Amy	Research Associate II
Prazich, Kathleen	Research Assistant
Rigby, Sean	Research Assistant
Rush, Brittany	Intern
Seitz, Brianna	Intern
Slavik, Julianna	Research Associate II
Trostle, Lynn	Grant Program Coordinator
Voeghtly, Laura	Postdoctoral Fellow
Weise, Jonathan	IT Support Specialist
Woznick, Lauren	Intern
GENEVA FOUNDATION PERSONNEL (Subaward)	
Chellappa, Mary E.	Research Coordinator/ Program Officer
Fonda, Stephanie	Sr. Research Scientist
Salkind, Sara	Program Officer
Schmidt, Virginia	Program Manager
Walker, Susan	Associate Investigator

Appendix B (Published Manuscripts)

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Early Empowerment Strategies Boost Self-Efficacy to Improve Cardiovascular Health Behaviors

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Abstract

Background: Self-efficacy, defined as confidence in the ability to carry out behavior to achieve a desired goal, is considered to be a prerequisite for behavior change. Self-efficacy correlates with cardiovascular health although optimal timing to incorporate self-efficacy strategies is not well established. We sought to study the effect of an empowerment approach implemented in the introductory phase of a multicomponent lifestyle intervention on cardiovascular health outcomes.

Design: Prospective intervention cohort study

Methods: Patients in the Integrative Cardiac Health Project Registry, a prospective lifestyle change program for the prevention of cardiovascular disease were analyzed for behavioral changes by survey, at baseline and one year, in the domains of nutrition, exercise, stress management and sleep. Self-efficacy questionnaires were administered at baseline and after the empowerment intervention, at 8 weeks.

Results: Of 119 consecutive registry completers, 60 comprised a high self-efficacy group (scoring at or above the median of 36 points) and 59 the low self-efficacy group (scoring below median). Self-efficacy scores increased irrespective of baseline self-efficacy but the largest gains in self-efficacy occurred in patients who ranked in the lower half for self-efficacy at baseline. This lower self-efficacy group demonstrated behavioral gains that erased differences between the high and low self-efficacy groups.

Conclusions: A boost to self-efficacy early in a lifestyle intervention program produces significant improvements in behavioral outcomes. Employing empowerment in an early phase may be a critical strategy to improve self-efficacy and lower risk in individuals vulnerable to cardiovascular disease.

Keywords: cardiovascular diseases; health behavior; lifestyle; prevention; risk factors; risk reduction; self-efficacy

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in Westernized nations (World Health Organization, 2010). Patients living with CVD experience decreased quality of life (Lewis et al., 2014), increase their utilization of health care resources (Tung et al., 1999) and decrease their economic productivity (Meland, Grønhaug, Oystese, & Mildestvedt, 2011). CVD prevention has therefore become a major goal of health care systems and medical professional societies (Eckel et al., 2014).

The main strategy in CVD prevention is to identify and improve risk factors (Eckel et al., 2014). Sustained improvements in CVD risk reduction requires that patients be made aware of their individual risk factors as well as their lifestyle behaviors that affect those risk factors such as avoiding tobacco use, making healthful nutrition choices, getting adequate exercise, managing stress levels, and getting adequate quantity and quality of sleep.

However, awareness alone is not adequate to change lifestyle behaviors affecting CVD risk (Elis et al., 2008), (Scotto, Waechter, & Rosneck, 2011). For behavior change, social-cognitive theory has proposed the concept of self-efficacy, defined as a person's belief in his/her ability to carry out behavior to achieve a desired goal (Bandura, 1977). A number of studies have shown that self-efficacy is a prerequisite for making behavioral changes for the self-management of chronic conditions such as hypertension (Criswell, Weber, Xu, & Carter,

2010), (Warren-Findlow, Seymour, & Brunner Huber, 2012), overweight (Linde, Rothman, Baldwin, Jeffery, 2006), (Roach, et al., 2003), and addictions (Kadden & Litt, 2011).

Integral to a collaborative care model for chronic disease is patient empowerment, which is defined as helping patients to develop the inherent capacity to be responsible for one's own life (Funnell & Anderson, 2003). Empowerment approaches include interactive teaching strategies designed to involve patients in problem solving and as a result impact self-efficacy. Although studies support the utility of this approach, health professionals need a way to operationalize the empowerment of patients (Anderson & Funnell, 2005). To lower CVD risk and improve adherence to healthy lifestyle change, strategies must be implemented to empower patients by enhancing self-efficacy.

There is an inverse relationship between self-efficacy and CVD risk factor profiles (Bailey, Kashani, Eliasson, & Vernalis, 2013), (Eliasson et al., 2015). However, the importance of self-efficacy for the management of CVD risks is not well established. Prior studies on this patient have shown mixed results. An observational study showed strong associations of high self-efficacy and adherence to two of four healthful behaviors for CVD (Sol, van der Graaf, van Peterson, & Visseren, 2011). A sub-analysis of a large prospective trial for treatment of hypertension showed that self-efficacy scores could not predict behavior change (Wingo et al., 2013). One randomized trial showed a lack of power for self-efficacy to predict adherence to 6 of 9 healthful behaviors (Sol, van der Graaf, van der Bijl, Goessens, & Visseren, 2008) and a second randomized trial showed equivocal results of an intervention to increase self-efficacy for exercise in cardiac rehabilitation patients (Barkley & Fahrenwald, 2013). Little is known about the appropriate timing or mechanism for the implementation of self-efficacy enhancing strategies to achieve successful behavior change.

In the present study, we investigated the impact of an intervention designed to enhance self-efficacy by giving patients an early boost using an empowerment approach to improve adherence to healthy lifestyle behaviors. The empowerment intervention was implemented as part of a cardiovascular (CV) health program targeting behaviors in the areas of nutrition, exercise, perceived stress and sleep.

2. Methods

The Integrative Cardiac Health Project (ICHP) is a prospective registry of patients enrolling in a 12-month CV health program. The study has been registered with clinicaltrials.gov and may be found using identifier NCT01975181. All patients give informed consent for participation in the registry and the study is being conducted according to the principles stated in the Declaration of Helsinki.

Patients are self-referred or referred by a healthcare provider to assess their CVD risks and to learn how those risks can be improved through lifestyle behavior changes. Patients participating in ICHP are men and women over 17 years of age who are eligible for care in the Department of Defense Healthcare System. Participants are comprised of active duty service members, dependents of service members, and retirees from active service along with their dependents. As such, the participants in ICHP include both genders with a broad spectrum of ages, races and ethnicities. Some patients entering ICHP have diagnosed coronary heart disease but the large majority is seeking to reduce CV risk factors.

Upon entry to ICHP, patients meet with a nurse practitioner (NP) to undergo a CV-focused history and physical examination and submit a cardiac-relevant laboratory panel of tests. Based on this baseline assessment, patients are categorized as low, intermediate, or high risk for CVD by the Framingham Risk Score, the most widely used CVD risk estimator. Family history of premature CVD was collected and defined as a parent or sibling who had a CV event before the age of 55 in men and 65 in women. Patients also complete a series of validated questionnaires to determine their individual pattern of lifestyle behaviors.

Specific questionnaires focus on the domains of the program and are administered at baseline and at program completion, at 12 months: nutrition (Rate Your Plate), exercise (minutes of continuous exercise per week), stress (Perceived Stress Scale), and sleep (Pittsburgh Sleep Quality Index, Fatigue Visual-Analog Scale). A CV-relevant Self-Efficacy Questionnaire is administered at baseline and after an empowerment workshop, at 8 weeks from baseline (See Table 1).

Table 1. Time Points for Program Milestones

	Time 1 (Baseline)	Time 2 (8 Weeks)	Time 3 (12 Months)
Framingham Risk Calculation	X		
Self-Efficacy Questionnaire	X	X	
Empowerment Intervention	X	X	
Rate-Your-Plate Nutrition Score	X		X
Exercise Minutes per Week	X		X
Perceived Stress Scale	X		X
Pittsburgh Sleep Quality Index	X		X
Fatigue Score	X		X

Self-Efficacy Questionnaire: Self-efficacy was measured with the adapted diabetes mellitus type 2 self-efficacy scale. Since most self-management tasks apply generally to chronic diseases as a whole, this scale was used to measure the level of confidence people have about their ability to perform the self-management tasks necessary to reduce vascular risk. The 9-item questionnaire is scored on a 5-point Likert scale, with a higher self-efficacy score corresponding with better self-efficacy. Reliability of the questionnaire was tested with a Cronbach's alpha of 0.69 (Sol, van der Graaf, van der Bijl, Goessens, & Visseren, 2006).

Framingham Risk Score: Cardiovascular disease risk was calculated using the standard FRS Hard Coronary Heart Disease (10-Year Risk) (National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002). The tool, which currently forms the foundation of current primary prevention guidelines, uses age, gender, total cholesterol, HDL cholesterol, smoking history and blood pressure (BP) to calculate the risk of coronary heart disease outcomes (MI and coronary death) over the subsequent 10 years. Scores are categorized as low ($\leq$10), medium (11-19) and high ($\geq$20).

Rate Your Plate (RYP): This ICHP-modified 26-item nutrition questionnaire, originally developed in 1983 by the Pawtucket Heart Health Program (PHHP), consists of questions focusing on foods that contribute the most fat, saturated fat, and cholesterol to the American nutrition. In a calibration study, the RYP was compared with the widely used Willet food frequency questionnaire (FFQ). When the RYP was administered prior to the Willet FFQ, Pearson product-moment correlations ranged between -0.45 and -0.65 on fat variables and nutrition cholesterol ($p < .001$ for all correlations), thus having the capacity to quantitatively reflect intake of fat and saturated fat (Gans, Hixson, Eaton, & Lasater, 2000), (Gans et al., 1993). The RYP individual score can indicate whether the participant's typical eating pattern is relatively high or low in fat, saturated fat and cholesterol. This questionnaire has been modified over the years to reflect changing national nutrition recommendations, fat-reduced foods now available in the marketplace, eating out and consideration of trans fatty acids in recommendations for spreads and cooking oils including the ICHP modifications to reflect use of beer, wine, alcohol, soda and other sugary drinks. Scores range from 26-78 with 26-42 reflecting least "heart healthy"; 43-60 middle ground, and; 61-78 most "heart healthy".

Perceived Stress Scale: PSS-14 developed in 1983 (Cohen et al., 1983) is one of the most widely accepted of measurements of stress (Cohen, Kamarck, & Mermelstein, 1983). Validation studies show that the PSS-14 has an internal consistency reliability of 0.85 by Cronbach alpha and a test-retest reliability of 0.85. This 14-item questionnaire asks the patient how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0 to 56 with higher scores correlating with higher stress. The PSS is designed for use with community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Scores in the low 20's reveal moderate stress levels while scores approaching 30 are substantial and concerning.

Pittsburgh Sleep Quality Index: PSQI is a self-rated questionnaire used to assess sleep quality and disturbances over a 1-month time interval (Buysse, Reynolds 3rd, Monk, Berman, & Kupfer, 1989). Nineteen individual items generate seven component scores whose sum yields one global score with a range of 0 to 21. The psychometric and clinical properties of the PSQI suggest its utility both in clinical practice and research

activities. A PSQI score greater than 5 has a diagnostic sensitivity of 89.6% and specificity of 86.5% (kappa = 0.75, $p \leq 0.001$). Essentially, a global score of greater than 5 indicates a poor sleeper. Sleep perturbations can be categorized by scores as follows: 0 to 5 is a good sleep score; 6 to 10 shows mild sleep difficulty; 11 to 15 moderate sleep difficulty, and 16 to 21 severe sleeping difficulty.

Fatigue Visual Analog Scale: This scale is borrowed from the Stanford Patient Education Research Center where it was tested on 122 patients, with mean value of 4.89 and standard deviations of 2.71 (Stanford Patient Education Research Center). This fatigue scale asks patients to express their experience of fatigue from 0 to 10 for the previous 2 week period. Patients who circle 5 to 6 express mild fatigue, 7 to 8 moderate fatigue, and 9 to 10 severe fatigue.

Empowerment Intervention: Empowerment strategies for patients are comprised of a comprehensive risk assessment report with detailed lifestyle recommendations for optimizing risk reduction generated by the NP. After two initial NP appointments which allow patients to problem-solve barriers to lifestyle change, clarify goals and identify motivations, patients attend a multi-disciplinary educational workshop. The workshop is comprised of an interactive healthy food demonstration and stress management experience and focus on the impact of actionable behaviors on health. For the remainder of the program and after the workshop, patients receive ongoing motivational advice by health coaches in order to achieve healthy goals in the program's domains as established by the NP in the first two weeks.

Statistics: Sample size calculation indicated that a sample of 100 participants would provide a stabilized and generalizable set of data. Plans were made to enroll approximately 115 patients to allow for a 15 percent rate of drop-out for failure to complete surveys properly. Data are presented as means with standard deviations or proportions. A median score was used to split patient Self-Efficacy Scores into two groups of nearly equal size allowing comparisons between high and low scoring groups. Demographic and survey health variables were compared using chi-square test for categorical variables, and Student's t-test for continuous variables. Pearson product-moment correlation coefficients were used to measure correlations because the data sets were normally distributed as seen on inspection of population histograms. All tests assumed $p < 0.05$ as statistical significance.

3. Results

The study population is comprised of 119 consecutive graduates of the ICHP CV program. The demographic variables of the group are provided in Table 2. The population is generally late middle aged, evenly split between men and women, representative of a variety of races, predominantly married and living in a family unit. Patients had an average of 2.5 CVD risk factors each, thus comorbid illnesses were common. Of the 119 patients, 9 (8%) were diagnosed with coronary heart disease, 67 (66%) with dyslipidemia, 61 (51%) with hypertension, 39 (33%) with obstructive sleep apnea, 32 (27%) with depression, 9 (8%) with diabetes, and 25 (21%) with pre-diabetes. Family history of premature CVD was reported by 53 patients (45%) in the total group. There were 20 (17%) patients who served as caregivers for other family members at home.

Table 2. Demographic variables for participants in the ICHP Registry

		All Patients (n = 119)	Low Self-Efficacy* (n = 59)	High Self-Efficacy** (n = 60)	p value***
Age (Years ± SD)		56.5 ± 13.1	55.2 ± 13.7	57.8 ± 12.6	0.28
Sex# men (%)		57 (48)	28 (47)	29 (48)	0.92
Race	White	85	41	44	0.85
	Black	21	12	9	
	Hispanic	5	3	2	
	Asian	2	1	1	
	Other	6	2	4	
Marital Status	Single	10	8	2	0.18
	Married	94	45	49	
	Divorced	11	5	6	
	Separated	4	1	3	

Number of Children	One	25	14	11	0.54
	Two	13	6	7	
	Three	39	18	21	
	Four or More	8	2	6	

*Low Self-Efficacy is defined as the group scoring below the median score of 36 points.

** High Self-Efficacy is defined as the group scoring at or above the median score of 36 points.

***p value denotes statistical difference between Low and High Self-Efficacy Groups by t-test for age and by chi square test for other variables.

Inspection of a histogram of the self-efficacy scores revealed that these were normally distributed. The median score (36 points) of the self-efficacy questionnaire measured at entry to ICHP was used to divide the participants into high (n=60) and low scorers (n=59). Demographic variables were not different for high and low self-efficacy subgroups (See Table 2). Framingham risk scores were calculated for each patient showing that nearly one third of patients were at intermediate or high risk for a CVD event over 10 years. Low self-efficacy patients were at higher CVD risk than high self-efficacy patients by Framingham estimation (See Table 3).

Table 3. At baseline, low Self-Efficacy correlates with higher CVD risk

	Medium or High Risk by Framingham	p value*
Low Self-Efficacy (n=59)	36%	0.04
High Self-Efficacy (n=60)	22%	

*Chi square analysis shows a significant difference between groups.

At baseline, the low self-efficacy group entered the ICHP program with lower scores for a healthy nutrition, less exercise minutes per week, higher levels of perceived stress, poorer sleep quality and greater fatigue (See Table 4).

Table 4. Change in Outcomes from Baseline to Completion According to Self-Efficacy Score

		All Patients (n = 119)	Low Self-Efficacy* (n = 59)	High Self-Efficacy** (n = 60)	p value***
Self-Efficacy (of 45 points)	Baseline	34.5 ± 6.5	29.1 ± 0.8	39.9 ± 3.0	NA
	Completion	40.3 ± 4.2	38.2 ± 4.6	42.4 ± 2.2	< 0.001
	Change	5.8, p<0.001	9.1, p<0.001	2.5, p<0.001	< 0.001
Nutrition (of 78 points)	Baseline	61.7 ± 8.3	58.9 ± 4.1	64.5 ± 7.4	< 0.001
	Completion	67.1 ± 6.0	65.7 ± 6.6	68.6 ± 5.0	0.008
	Change	5.4, p<0.001	6.8, p<0.001	4.1, p<0.001	0.01
Exercise (minutes per week)	Baseline	156 ± 125	110 ± 87	201 ± 141	< 0.001
	Completion	220 ± 163	186 ± 157	253 ± 163	0.02
	Change	64, p<0.001	76, p=0.002	52, p=0.06	0.16
Perceived Stress (of 56 points)	Baseline	20.1 ± 9.1	22.0 ± 8.5	18.3 ± 9.3	0.02
	Completion	17.2 ± 8.6	18.3 ± 8.7	16.1 ± 8.4	0.16
	Change	2.9, p=0.01	3.7, p=0.02	2.2, p=0.18	0.18
Sleep Quality (of 21 points)	Baseline	7.1 ± 3.9	7.9 ± 4.3	6.2 ± 3.2	0.02
	Completion	4.7 ± 3.5	5.3 ± 4.1	4.1 ± 2.7	0.06
	Change	2.4, p<0.001	2.6, p=0.001	2.1, p<0.001	0.53

Fatigue (of 10 points)	Baseline	4.3 ± 2.5	5.0 ± 2.4	3.6 ± 2.3	0.001
	Completion	3.0 ± 2.2	3.2 ± 2.3	2.9 ± 2.1	0.39
	Change	1.3, p<0.001	1.8, p<0.001	0.7, p=0.07	0.01

*Low Self-Efficacy is defined as the group scoring below the median score of 36 points.

** High Self-Efficacy is defined as the group scoring at or above the median score of 36 points.

***p value denotes statistical difference by t-test between Low and High Self-Efficacy Groups.

These findings were corroborated with Pearson r product-moment correlations which showed a strong correlation of nutrition scores and moderately strong correlations of exercise minutes, lower stress scores, and better sleep quality with total self-efficacy scores (See Table 5).

Table 5. At baseline, improvements in Self-Efficacy Score correlate with improvements in health indices.

	Nutrition Score	Exercise Minutes	Stress Levels	Sleep Quality
Total SE Score	0.47	0.37	0.30	0.36
	(p<0.001)	(p<0.001)	(p=0.03)	(p<0.001)

The Pearson r coefficients show a strong correlation between baseline self-efficacy score and nutrition score and moderately strong correlations for exercise, stress and sleep.

In response to the empowerment intervention of the ICHP program, 98 of the total 119 patients (82%) showed gains in self-efficacy with an average improvement of 7.2 ± 4.4 points; 11 (9%) showed no change; and 10 (8%) decreased their self-efficacy an average of 2.0 ± 1.2 points. In the group of 59 patients with low self-efficacy at program entry, 58 (98%) showed improvements averaging 9.4 ± 4.4 points in self-efficacy and only one (2%) decreased self-efficacy by 2.0 points.

Among all 119 participants, only three (3%) were active tobacco smokers, each reporting current smoking of 2 cigarettes per day. Nineteen other patients were former smokers, having an average 1 pack per day history of smoking, and having quit an average of 26 years prior. Since tobacco use occurred so infrequently in the study population, further analysis of tobacco use was not performed.

4. Discussion

The salient finding of the current study is that a boost to self-efficacy early in a lifestyle intervention program produces substantial improvements in behavioral outcomes. The overwhelming majority of patients responded with improved self-efficacy scores. Self-efficacy scores increased irrespective of baseline self-efficacy.

Though patients in both the low and the high self-efficacy groups showed improvements in self-efficacy and behavioral survey scores, the largest gains in self-efficacy occurred in patients who ranked in the lower half for self-efficacy at baseline. This lower self-efficacy group also demonstrated behavioral improvements that erased differences between the high and low self-efficacy groups or at least provided a substantial "catch-up" such that the scores on completion of the program were approaching or better than baseline scores for the high self-efficacy group.

Our findings agree with prior reports that self-efficacy scores at baseline correlate with the cardiovascular risk profile. Indeed in our population, patients with low self-efficacy scores were found to have a higher predicted cardiovascular risk by Framingham Risk Score in addition to less healthy cardiovascular behaviors (Table 3). Given the burden of CV risk and comorbid illness in the low self-efficacy group it is critical to provide self-care behavioral tools to overcome lifestyle behavioral change barriers.

Prior studies assessing the impact of self-efficacy on adherence to behavior change have frequently been limited to a single behavioral dimension such as nutrition (Sharp & Salyer, 2012), (Timlin, Shores, & Reicks, 2002), (Nothwehr, 2008), (Cha, 2014) or exercise (Slovinec D'Angelo, Pelletier, Reid, & Huta, 2014), (Schwarzer, Luszczynska, Ziegelmann, Scholz, & Lippke, 2008). Investigations evaluating the impact of a self-efficacy intervention on multiple behaviors have had mixed results. One randomized controlled trial reported positive effects on nutrition and exercise but not on smoking and alcohol intake (Sol et al., 2008). Another prospective cohort study showed the beneficial effect of increased self-efficacy on nutrition, exercise and stress management

(Clark & Dodge, 1999). The present prospective cohort study shows positive effects of improved self-efficacy on nutrition, exercise, and stress management behaviors but extends the positive effects to sleep improvement as well.

Employing an empowerment intervention early in the sequence of events in a heart-healthy program provides a mechanism for increased patient self-efficacy. Our findings validate numerous studies showing that interventions that aim to empower patients are valuable in promoting patient well-being, decision-making and self-management of chronic disease (Aujoulat, Marcolongo, Bonadiman, & Deccache, 2008).

The results of the current study appear to be generalizable to other locations and institutions. This group of patients at risk for CVD mirrors the population at large with regard to demographic profile and comorbid illnesses. The intervention that was provided does not require special equipment or resources and is therefore scalable and could be duplicated in other centers targeting CVD prevention.

The current study has limitations. Because the intervention aimed at multiple dimensions of healthy CV behaviors, it is not possible to determine which aspects of the empowerment intervention were most effective. Likewise, with the current study design it is not possible to determine whether or not there was a synergistic impact from improvement in one behavior that helped stimulate improvements in other behaviors. A second limitation is the use of measurement tools that rely on self-report. While these tools are validated instruments to measure the behaviors for which they were targeted, the use of objective measures would give more robust information and therefore be more convincing. Unfortunately, objective measures are complex (nutrition measures), unwieldy (actigraphy for exercise and sleep), or do not readily exist (stress management).

In summary, the results of this study support the idea that a lifestyle behavioral change program aimed at providing an early boost to self-efficacy is feasible and can yield positive results. These findings are particularly significant in high-risk patients who are vulnerable to CVD and may be in a position to make critical behavioral lifestyle modifications to lower their risk of overt disease. Further study is warranted to measure the impact that such behavior changes have on prevention of CVD events.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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A Systematic Approach Incorporating Family History Improves Identification of Cardiovascular Disease Risk

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Background: Although family history (FH) is an independent predictor of cardiovascular disease (CVD) risk, traditional risk scores do not incorporate FH. Nurse practitioners routinely solicit FH but have no mechanism to incorporate the information into risk estimation. Underestimation of risk leaves clinicians misinformed and patients vulnerable to the CVD epidemic. **Objective:** We examined a systematic approach incorporating FH in CVD risk assessment, validating risk reclassification using carotid intima-media thickness (CIMT), a surrogate measure of atherosclerosis. **Methods:** Of 413 consecutive patients prospectively enrolled in the Integrative Cardiac Health Project Registry, a subgroup of 239 was low or intermediate risk by the Framingham Risk Score. A systematic approach for the assessment of FH was applied to this subgroup of the registry. A positive FH for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, conferred reclassification to high risk. Reclassification was validated with CIMT results. **Results:** Chart audits revealed adherence to the systematic approach for FH assessment in 100% of cases. This systematic approach identified 115 of 239 (48%) patients as high risk because of positive FH. Of the reclassified patients, 75% had evidence of subclinical atherosclerosis by CIMT versus 55% in the patients not reclassified, $P < 0.001$. Logistic regression identified positive FH for premature CVD (odds ratio, 2.6; $P = 0.001$) among all variables, as the most significant predictor of abnormal CIMT, thus increasing risk for CVD. **Conclusions:** The Integrative Cardiac Health Project systematic approach incorporating FH into risk stratification enhances CVD risk assessment by identifying previously unrecognized high-risk patients, reduces variability in practice, and appropriately targets more stringent therapeutic goals for prevention.

KEY WORDS: cardiovascular disease, family history, primary prevention, risk assessment

Cardiovascular disease (CVD) is the leading cause of death and disability in the United States and Europe.[1,2] On the basis of numerous analyses performed to determine the thresholds for increased risk, family history (FH) of premature CVD is defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women.[3–12] With this definition, FH of premature CVD is an independent and robust predictor of risk. When FH is positive, individual risk for CVD is increased by as much as 5-fold.[10] Although US and European guidelines include positive FH as a high-risk factor, traditional risk scoring systems do not. Nurse practitioners routinely inquire about FH in clinical practice, but there is variability in the approach to capture and interpret the data.[5,13,14]

The Framingham Risk Score (FRS), the most widely used CVD risk assessment tool, significantly underestimates risk because it does not incorporate FH data.[15,16] Studies show FRS to be only 50% accurate in identifying patients at high risk for heart disease.[15] In fact, up to 75% of patients experiencing an acute coronary syndrome are assessed as low risk by the FRS.[17] When FH is not used in risk assessment, a large subgroup of the population at risk for CVD remains unrecognized, leaving them unaware of their threatened health status. Failing

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to identify these high-risk individuals precludes clinicians from prescribing targeted and risk-specific self-care interventions aimed at CVD prevention.[13]

Although FH has been repeatedly demonstrated to be a high risk factor of CVD, current guidelines provide no mechanism for the systematic collection, interpretation, and risk score adjustment using this information. We implemented a systematic approach for the assessment of FH to standardize identification of high-risk patients and used carotid intima-media thickness (CIMT) to validate the high-risk reclassification.[18,19]

Methods

This investigation was conducted with the approval of the institutional review board at Walter Reed National Military Medical Center in Bethesda, Maryland. The study design is a subgroup analysis of data prospectively collected on consecutive patients enrolled in the Integrative Cardiac Health Project (ICHP) Registry. The ICHP Registry is a CVD prevention program operating in a research Center of Excellence for the US Department of Defense. All subjects gave informed consent for participation in the registry, and the study was conducted according to the principles stated in the Declaration of Helsinki.

The ICHP offers military healthcare beneficiaries a 6-month tailored CVD risk reduction program. Patients who join the program by self or provider referral must be adults older than 17 years. All patients seen at the ICHP are categorized upon baseline assessment as low, intermediate, or high risk for CVD by the FRS. In addition, ICHP patients receive results of a detailed CVD risk assessment and a personalized preventive health plan. As part of the ICHP Registry, patients receive a CIMT, which is maintained as a long-term CVD outcome measure. The CIMT findings are not used to calculate the patient's CVD risk status. The following variables were collected on all patients who attended the ICHP from 2008 to 2011: age, gender, ethnicity, FRS, FH status, CIMT and diagnoses of CVD, hypertension, dyslipidemia, and diabetes.

Upon entry to the ICHP, patients undergo a cardiovascular-focused history and physical examination. Medical history, including smoking history, is elicited with a written question as part of a questionnaire, and the responses are verified verbally by a nurse practitioner at the time of the physical examination. medical history such as hypertension, diabetes, and dyslipidemia is also elicited on the questionnaire, validated verbally by a nurse practitioner and reconciled with data recorded in the patient's medical record. Body mass index (BMI) is calculated with the formula kilograms divided by the square of height in meters using measured height and weight from a medical-grade weight scale and stadiometer. Blood pressure is first measured after the patient has been sitting quietly for 5 minutes using a GE DINAMAP PRO Series 100–400V2. Five minutes later, a second blood pressure reading is taken, and the 2 values are averaged for the record. All cardiovascular-relevant laboratory data are obtained in the blood chemistry laboratory at the medical facility, with the laboratory certified by the Clinical Laboratory Improvement Amendments.

At a subsequent appointment, the patients were informed of their CVD risk status and were provided therapeutic goals specific to their determined risk category. Although the patients in all risk categories (low, intermediate, and high) received recommendations for healthy behavior change, the high-risk patients were targeted with aggressive treatment goals for cholesterol, blood pressure, and weight management.

This analysis was limited to a subgroup of ICHP patients whose calculated FRS showed low or intermediate 10-year risk because the high-risk patients could not be reclassified to a higher level of risk. Diabetes is considered by the FRS to be a high-risk factor, and therefore, any patient with diabetes was excluded from this analysis.

Risk Assessment (Carotid Intima-Media Thickness)

The CIMT findings were reviewed and evaluated by 1 sonographer oriented to the purposes of the project but blinded to the FH information for each patient. Images were obtained on a single ultrasound machine (SonoSite MicroMaxx 3.4.3; Bothell, Washington) using a linear array 5- to 10-MHz transducer with standardized image settings, including resolution mode, depth of field, gain, and transmit focus. All sonograms were obtained with the patients supine with the head facing the contralateral side. Electrocardiograms were recorded simultaneously. The sonographer, also trained in the measurement of CIMT, performed the analyses with commercially available software (Sonocalc IMT, Bothell, Washington). Carotid intima-media thickness was determined from images of the far wall of the distal common carotid arteries (immediately proximal to the carotid bulb) and reported as the mean value for the bilateral measurement. The near (intimal-luminal interface) and far (medial-adventitial interface) field arterial wall borders were manually traced for measurement of mean CIMT (millimeters) across a 10-mm arterial segment. A mean CIMT measurement of greater than the 75th percentile cutoff value, based on age and gender, in at least 1 carotid vessel was defined as an abnormal CIMT, as proposed by the American Society of Echocardiography Carotid Intima-Media Thickness Task Force.[20] This cutoff value has been used in a prior large atherosclerosis outcomes study, the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) Study, with CIMT as its main outcome measure.[21]

Impact Assessment

For CVD risk assessment, ICHP nurse practitioners evaluated FRS and FH status. The FRS, which takes into account age, gender, smoking, systolic blood pressure, total cholesterol, and high-density cholesterol levels, was determined using a web-based tool.[22] A systematic approach to evaluating FH was applied to standardize risk stratification beyond the FRS (see Figure). The ICHP nurse practitioners were trained using a standardized operating procedure (SOP) detailing the collection of FH during the initial assessment of each patient. This SOP defined positive FH of premature CVD as a first-degree relative (parent or sibling) having a CVD event before the age of 55 years in men and 65 years in women.[11,12] Cardiovascular disease events included myocardial infarction; cardiovascular revascularization; and diagnosis of coronary disease, stroke, or transient ischemic attack. The family tree was explored in detail for these CVD events, specifically in first-degree relatives and for the age of occurrence. Any first-degree family member meeting these criteria conferred a high-risk designation irrespective of the FRS result. Patients who were unable to provide FH (for example, patients who are adopted and do not have FH information) were excluded from the analysis. Chart audits were performed on 100% of cases to verify adherence to the systematic approach outlined in the SOP.

Analyses were performed using the Statistical Package for the Social Sciences (version 20.0).[23] Descriptive and frequency statistics were presented as mean (SD) or percentage. Student t test for continuous variables and χ^2 analysis for categorical variables were used. Logistic regression was performed to assess the predictive impact of factors on the likelihood of a patient having an abnormal CIMT.

Results

Of 413 patients, 19 patients (4.6%) were excluded for lack of FH data, leaving 394 for this analysis. Using the FRS, 239 of 394 patients (61%) were classified as low or intermediate risk. Frequency and descriptive analyses revealed a normally distributed population by age with no missing data. Demographic findings showed a mean age of 49 years (range, 20–76); 59% were women; 51%, white; 25%, black; 6%, Hispanic; and 1%, Asian, with 17% undeclared or other. The mean body mass index was 30.5 kg/m^2. The population was characterized by hypertension (40%), dyslipidemia (71%), and smoking (2%).

Chart audits revealed adherence to the systematic approach for FH assessment in 100% of the 239 patients who were in the low or intermediate FRS category. The systematic approach identified 115 of 239 patients (48%) as having positive FH for CVD. Table 1 displays the comparison between the 2 groups (positive FH and negative

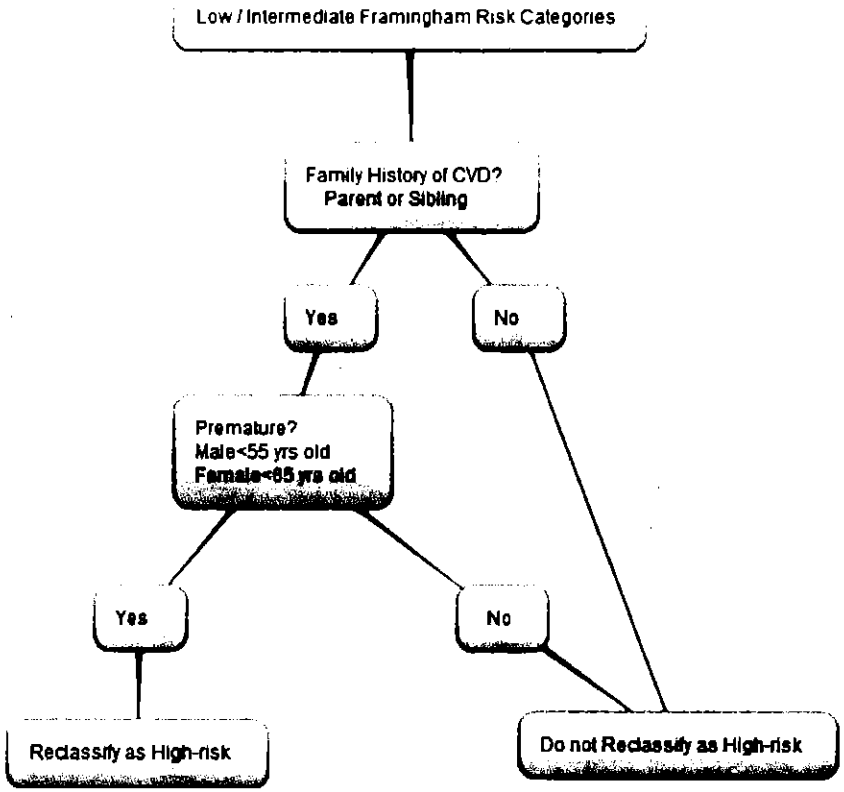

FIGURE. *The Integrative Cardiac Health Project systematic approach incorporating family history in CVD assessment.*

TABLE 1 Baseline Characteristics of Population at Low and Intermediate Cardiovascular Disease Risk

N = 239	Negative FH, n = 125	Positive FH, n = 114	*P*
Age, y	44.9 (12.18)	54.3 (10.16)	0.02[a]
Gender (female)	55%	64%	0.17
BMI, kg/m^2	29.5	31.3	0.39
Active smoker	3%	2%	0.86
Hypertension	31%	39%	0.18
Dyslipidemia	74%	70%	0.47
FRS	3.01 (3.21)	4.5 (4.19)	0.001[a]
Glucose, mg/dL	89.8 (10.1)	92.8 (9.68)	0.84
CIMT (abnormal)	55%	75%	<0.001[a]

Data are presented as mean (SD) or percentage. *t* test is used for continuous variables. χ^2 analysis is used for categorical variables. *P* values are given for the comparison between FH groups.
[a]Denotes statistical significance.

FH). Between FH groups, age, FRS, and CIMT were different. The patients with a positive FH were older (54.3 vs 44.9 years, $P = 0.02$). The mean FRS scores were statistically different (positive FH, 4.5; negative FH, 3.0; $P < 0.001$), although this difference is not clinically important because both scores indicate low risk. In validating the reclassification using CIMT, the proportion of patients with an abnormal CIMT was clinically and statistically different between groups, with a higher percentage in the positive FH group (75% vs 55%, $P < 0.001$). No effect of confounding was detected because there was no difference between groups using χ^2 analysis for gender, BMI, smoking history, hypertension, and dyslipidemia.

Logistic regression was performed to assess the impact of factors on the likelihood that patients would have an abnormal CIMT (Table 2). The model contained 5 independent variables (race, gender, FH category, diagnoses of hypertension and dyslipidemia). Age was not included in the model because age is one of the normative factors used as a cutoff value in the definition of normal versus abnormal CIMT.[20] The full model containing all predictors was statistically significant, χ^2 (11, n = 239) = 41.1, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT. The model as a whole explains between 16% and 22% of the variance in CIMT status and correctly classified 69% of cases after inclusion of the predictors. Two of the independent variables made a unique statistically significant contribution to the model (black race: odds ratio [OR], 5.8; $P = 0.02$; 95% confidence interval [CI], 1.3–26.9, and presence of positive FH: OR, 2.4; $P = 0.006$; 95% CI, 1.3–4.5). In an effort to find the most parsimonious model predicting abnormal CIMT,[24] logistic regression was repeated using the 2 contributing variables, black race and presence of positive FH. This new model containing the 2 predictors was statistically significant, χ^2 (6, n = 239) = 28.6, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT. The model as a whole explains between 11% and 16% of the variance in CIMT status and correctly classified 69% of cases after inclusion of the predictors. Although black race was no longer a significant predictor in the new model, presence of positive FH remained the only significant predictor contributing to the logistic regression model (black race: OR, 0.528; $P = 0.290$; 95% CI, 0.162–1.725, and presence of positive FH: OR, 2.64; $P = 0.001$; 95% CI, 1.47–4.73). The Hosmer-Lemeshow test showed goodness of fit with a significance of 0.86.

Discussion

Although national guidelines recognize the importance of FH for CVD risk, these guidelines provide no mechanism to instruct practitioners on how to translate this FH information to a more accurate determination of risk for the individual patient.[1,2,5] In fact, there has been

TABLE 2 Logistic Regression Model

Predictors of Abnormal CIMT	*B*	SE	Wald	*df*	*P*	OR	95% CI for OR	
							Lower	Upper
Black race	1.761	0.781	5.088	1	0.024[a]	5.816	1.260	26.856
Gender	0.441	0.318	1.921	1	0.166	1.554	0.833	2.897
FH positive	0.883	0.318	7.691	1	0.006[a]	2.418	1.296	4.513
Diagnosis of hypertension	0.540	0.346	2.435	1	0.119	1.716	0.871	3.382
Diagnosis of dyslipidemia	0.196	0.347	0.320	1	0.572	1.217	0.616	2.404
Constant	−1.736	0.808	4.612	1	0.032	0.176		

The model contained 5 independent variables (race, gender, positive FH, diagnosis of hypertension, and diagnosis of dyslipidemia). The full model containing all predictors was statistically significant, χ^2 (11, n = 239) = 41.1, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT.
[a]Denotes statistical significance.

a call for evidence on the value of systematically using FH in CVD risk assessment.[5]

Investigation of FH requires a systematic approach in which there is minimized variability in assessment of risk among clinicians because there are numerous criteria needed to fulfill the definition of positive FH. These criteria are complex and require an in-depth review of the family tree including gender, relationship to the patient, and age of onset of CVD. A simple yes/no question is inadequate to provide the relevant data to illicit an accurate FH for risk estimation.[5]

Our study population of mostly overweight, late–middle-aged subjects with a variety of races is fairly typical of a population seeking medical evaluation for CVD risk estimation. One risk factor that makes our sample population stand out as different from the US population is the very low prevalence of self-reported smoking behavior (2%), which is substantially lower than US norms (19%).[25] A potential explanation for this discrepancy is that there have been initiatives for health promotion that champion smoking cessation, including a ban of smoking on site in the medical facility. Furthermore, self-referred patients seeking wellness in a CVD risk reduction program may also be less likely to smoke.

We have shown that, among asymptomatic, previously low- or intermediate-risk patients by FRS, the use of a systematic approach for the incorporation of FH resulted in identifying a substantial proportion of patients at high risk for CVD. These patients would have otherwise been told that they were not at high risk for CVD. In addition, we have demonstrated the feasibility of implementing a systematic approach for incorporating FH, an easily accessible and inexpensive data point.[26]

The validity of this reclassification was substantiated using CIMT in the positive FH group to find 75% abnormal CIMT results compared with 55% abnormality in the group with negative FH. This is consistent with findings from the Framingham Offspring Study, a large population-based cohort of families in which CVD events were validated prospectively in both parents and offspring.[11] On the basis of that study, an association was found between parental history and subclinical atherosclerosis among offspring measured by CIMT.

Our study highlights the predictive value of including FH in assessment of risk for CVD. By logistic regression, positive FH was shown to be a robust predictor, indicating that patients with presence of positive FH were more than twice as likely to have an abnormal CIMT compared with those with negative FH, when controlling for all other factors in our data set. Although positive FH was an independent predictor, other factors including age, race, gender, and diagnoses of hypertension and dyslipidemia were not predictors of an abnormal CIMT. This may be explained by an underlying atherosclerotic mechanism causing functional abnormalities in offspring of patients with premature CVD, independent of known vascular risk factors.[27–29]

The mean age of the patients with a positive FH was greater than of the patients with negative FH in our cohort. This finding may be explained by the fact that older study subjects will have older siblings who are more likely to have experienced a cardiovascular event and younger study subjects will more likely have younger siblings who have not yet developed CVD. The older sibling's event gives the older study subject a positive FH, whereas younger study subjects are more likely to have a negative FH.

The lack of a mechanism to incorporate FH in CVD risk assessment is a major gap in current practice. This article suggests a systematic approach to translate the evidence for FH into clinical practice. When patients at high risk for CVD are properly identified, they are given appropriate therapeutic goals to match their heightened risk category, and more attention is paid to healthy lifestyle behavior change. Ultimately, incorporating FH in risk assessment is a way to personalize preventive therapies aimed at combating the epidemic of CVD.

Limitations

Limitations include the use of CIMT as a surrogate measure for CVD events. However, this is a commonly used strategy to overcome expense, feasibility issues, and risk associated with radiological studies such as electron beam computerized tomography and computed tomographic angiography.[18]

Although our sample population shows some characteristics that mirror the US population generally such as overweight,[30] an important characteristic that deviates from the US population is the very low prevalence of smoking status (2%). This difference may limit our ability to generalize our findings to the population at large. Another potential limitation may be referral bias because patients with positive FH may have a heightened sense of concern regarding their CVD health before entering the program.

Furthermore, data collection did not include all individual variables thought to influence CVD, although variables necessary for FRS calculation were captured. A further limitation is that approximately 5% of our patients were unable to provide FH.

Conclusions

Translation of evidence into practice is dynamic, and mechanisms to help clinicians accomplish translation continue to evolve. Recent evidence indicates that positive FH has predictive validity.[4] This study demonstrates that a reproducible systematic approach for adding FH to current practice enhances predictive value and identifies high-risk patients who, at present, are not captured.

This report describes a mechanism that addresses a current gap in clinical practice. The findings of this report are sufficiently promising to warrant further implementation and validation in other settings, using different study designs and outcome measures.

What's New and Important

- Family history for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, confers a high-risk classification for CVD as validated by a surrogate marker of atherosclerosis.
- A systematic approach for incorporation of FH for premature CVD will enhance the identification of high-risk patients.
- Incorporating FH in risk assessment is a way to personalize preventive therapies aimed at combating the epidemic of CVD.

We urge practitioners to adopt a systematic approach to incorporate FH in CVD risk assessment to provide patients with more accurate risk stratification and to target preventive interventions for high-risk individuals. We believe that implementation of such a systematic approach would have a global impact on patients at risk for CVD.

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Original Article
EPIDEMIOLOGY/GENETICS

Obesity

Importance of Substantial Weight Loss for Altering Gene Expression During Cardiovascular Lifestyle Modification

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Objective: To examine relationships between weight loss through changes in lifestyle and peripheral blood gene expression profiles.

Methods: A prospective nonrandomized trial was conducted over 1 year in participants undergoing intensive lifestyle modification to reverse or stabilize progression of coronary artery disease. Cardiovascular risk factors, inflammatory biomarkers, and gene expression as a function of weight loss were assessed in 89 lifestyle participants and 71 retrospectively matched controls undergoing usual care.

Results: Substantial weight loss ($-15.2 \pm 3.8\%$) in lifestyle participants ($n = 33$) was associated with improvement in selected cardiovascular risk factors and significant changes in peripheral blood gene expression from pre- to post-intervention: 132 unique genes showed significant expression changes (false discovery rate corrected P-value <0.05 and fold-change ≥ 1.4). Altered molecular pathways were related to immune function and inflammatory responses involving endothelial activation. In contrast, participants losing minimal weight ($-3.1 \pm 2.5\%$, $n = 32$) showed only minor changes in cardiovascular risk factors and markers of inflammation and no changes in gene expression compared to non intervention controls after 1 year.

Conclusions: Weight loss ($\geq 10\%$) during lifestyle modification is associated with down-regulation of genetic pathways governing interactions between circulating immune cells and the vascular endothelium and may be required to successfully reduce CVD risk.

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Introduction

Data from the National Health and Nutrition Examination Survey indicate that 68% of adults in the United States (US) are overweight ($25 \leq$BMI< 30 kg/m^2) or obese (BMI≥ 30 kg/m^2) (1). Obesity is associated with significantly higher all-cause mortality in the general population (2) and is an independent risk factor for coronary artery disease (CAD) and myocardial infarction (MI) (3). If obesity continues to escalate at current rates, total healthcare costs attributable to obesity-related care could reach >$860 billion by 2030 and account for 18% of total healthcare expenditures in the US (4).

Lifestyle intervention has become an integral component of cardiovascular disease (CVD) risk reduction therapy because healthy lifestyle behaviors are effective for improving risk factors (5) and significantly reducing risk for MI (6). Weight loss in particular has been associated with positive changes in endothelial function (7)

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Author contributions: DE conceived the study and wrote the manuscript, KM and NC conducted statistical analysis of risk factor data, HB carried out experiments, FM and GJ conducted statistical analysis of gene expression data, RVL conducted pathways analysis, RE and MV were involved in writing the paper and providing clinical perspective.

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and markers of endothelial health (8); however, the amount of weight loss and/or the treatment regimen by which weight loss is achieved may have different metabolic effects. For example, modest weight loss of <10% over a short period of time may be sufficient to improve plasma lipid profiles and insulin sensitivity (9), but substantial long-term weight loss of ≥10% may be necessary to significantly modulate circulating biomarkers of inflammation (10) and make clinically meaningful improvements in vascular health (11).

Clinical studies have shown that gene expression in peripheral blood is associated with coronary heart disease (12) and atherosclerotic involvement (13), but the importance of weight reduction through lifestyle modification in modulating blood-based gene expression is not well known. Our previous research showed that significant changes in the expression of genes governing processes important to vascular health occur during lifestyle modification (14), but the physiological drivers of molecular change remain unknown. In this study, we examined changes in peripheral blood gene expression as a function of weight loss during a cardiovascular lifestyle intervention to better understand molecular mechanisms by which diet and exercise affect cellular processes involved in CVD risk reduction. We hypothesized that the amount of weight loss would affect changes in traditional CVD risk factors, inflammatory molecules, and patterns of gene expression, which may influence vascular physiology and health.

Methods

Participants and intervention

A prospective, nonrandomized clinical intervention, based on the Multicenter Lifestyle Demonstration Project, was used to promote weight loss and reduction of CVD risk factors through changes in lifestyle (15). To be eligible, prospective participants were required to have CAD diagnosed by a physician or to have 2 or more risk factors. Criteria for CAD included stable angina, angioplasty, evidence of ≥50% luminal narrowing on coronary angiogram, acute MI, bypass surgery, or stent placement; risk factors were obesity (BMI ≥30), hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), high total cholesterol (>200 mg/dL), physician diagnosed diabetes, or family history of heart disease in parents or siblings. Additional acceptance criteria included physician approval and motivation to follow the program guidelines for 1 year. All patients were required to abstain from tobacco use for at least 3 months prior to enrollment and throughout the program.

Participants were required to adopt, and strictly follow for 1 year, a low fat vegetarian diet (<10% of calories from fat) with emphasis on whole grains, fruits, and vegetables, practice 1 hour of stress management per day by doing progressive relaxation, yoga, or meditation, perform 3 hours of aerobic exercise each week such as walking, cycling, rowing, or aerobics, and attend weekly group support sessions. Clinical staff met with patients twice each week during the first 3 months to orient participants to the program and maximize adherence. The remainder of the program was primarily self-directed but included ongoing weekly stress management and group support sessions.

Controls were recruited prospectively and were matched to intervention participants based on gender, age at entry within a 5-year window, and disease status (CAD or risk factors). Control subjects received standard care from their primary physicians, but did not participate in any component of the lifestyle program or receive any information, advice, or counseling regarding healthy lifestyles. This study was conducted at Windber Research Institute; the protocol and consent form were approved by the Windber Medical Center Institutional Review Board. The study is registered as NCT01805492 at ClinicalTrials.gov.

Anthropometric measurements

Data collection and reporting followed recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group (http://www.cdc.gov/trendstatement/). Demographic and clinical information was obtained from participants and controls by standard questionnaires at the baseline and 1-year examinations. Height and weight were measured on a combined scale (Cardinal Scale, Webb City, MO, USA). Exercise capacity was determined by exercise intensity and duration during a graded treadmill exercise test.

Blood collection and plasma assays

Fasting blood samples were collected in the morning on the day of each examination and placed directly on ice. Within 2 hours of collection, whole blood was centrifuged at ~1300*g* for 10 min and plasma aliquots were stored at −80°C. Standard lipid assays were conducted using the AEROSET™ clinical chemistry system (Abbott Laboratories, Abbott Park, IL, USA). C-reactive protein (CRP), ultra-sensitive insulin, and leptin were measured in duplicate on freshly-thawed plasma samples by radioimmunoassay (EMD Millipore, Darmstadt, Germany) at the Johns Hopkins Bayview Clinical Research Unit. Intra-assay coefficients of variation (CV%) were 6.33 for CRP, 2.69 for insulin, and 3.85 for leptin.

Dietary composition

Participants and controls completed a self-reported 72-hour dietary recall questionnaire at each examination, recording their total intake for all meals and snacks over 3 consecutive days. Reports included specific food items and drinks consumed, portion sizes, and preparation methods. Daily caloric intake and nutrient composition were then determined using Food Processor® v10.10 (ESHA Research, Salem, OR, USA).

Gene expression analysis

Peripheral blood for gene expression analysis was obtained from participants and controls at each examination using the PAXgene™ Blood RNA System (Qiagen, Valencia, CA, USA). Globin mRNA transcripts were depleted from a portion of each RNA sample using the GLOBINclear™-Human kit (Life Technologies, Carlsbad, CA, USA). Globin-depleted RNA aliquots (1 μg) were amplified using the MessageAmp™ II aRNA Amplification System (Life Technologies) and the resulting double-stranded complementary DNA was *in vitro* transcribed to synthesize amplified RNA (aRNA). Aliquots of aRNA (15 μg) labeled with biotin-11-UTP were then purified, fragmented, and hybridized to GeneChip® Human Genome U133A 2.0 arrays (Affymetrix, Santa Clara, CA, USA) and scanned on a GeneChip® Scanner 3000. RNA samples from both time points for each participant were processed together in the same batch to minimize technical artifact. The raw gene expression data have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) and are accessible through GEO Series accession numbers GSE46097 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=

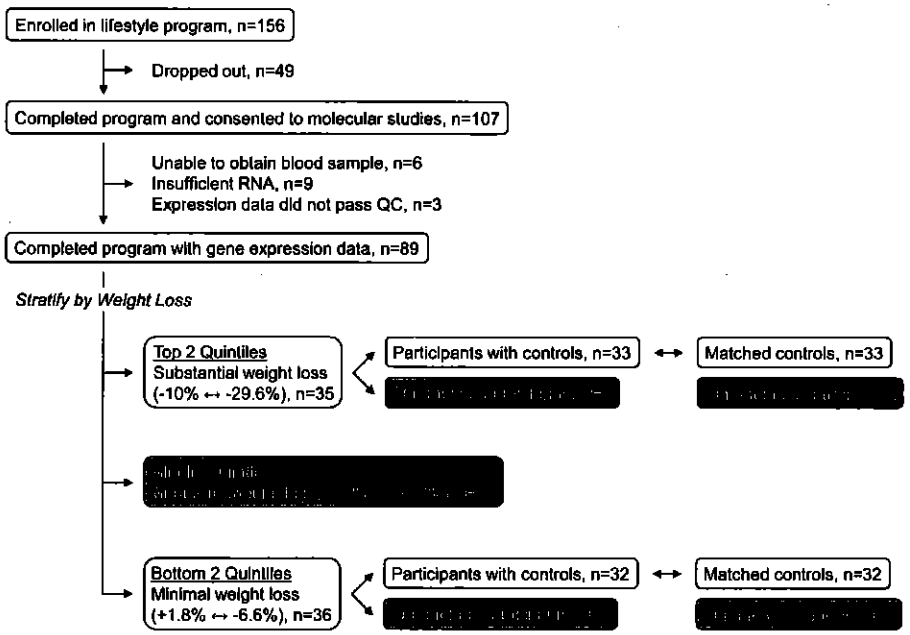

Figure 1 Flow diagram showing participant enrollment, attrition, and subgroup analysis.

GSE46097) and GSE66175 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66175).

Data analysis

Statistical analysis of traditional risk factors and biochemical variables was conducted using JMP® (v10.0). Lifestyle participants ($n = 89$) were stratified into quintiles based on weight loss over 1 year (Supporting Information Table S1). The top 2 quintiles were considered "substantial weight loss" while the bottom 2 quintiles were designated "minimal weight loss." The middle quintile ($n = 18$) with mean weight loss $-8.3 \pm 0.8\%$ was excluded from further analysis (Supporting Information Table S2). A flow diagram showing participant enrollment, attrition, and subgroup analysis is presented in Figure 1. For participants in the substantial ($n = 33$) and minimal ($n = 32$) weight loss groups with matched controls, baseline risk factor levels were then compared between participants and controls using a Wilcoxon Signed Rank nonparametric test for matched pairs. Differences in traditional risk factor response over time among the matched pairs were assessed with a matched-pairs t-test; changes in plasma biomarkers were compared between groups with a Wilcoxon Signed Rank nonparametric test.

The gene expression data were analyzed with Partek® Genomics Suite v6.5 (Partek Incorporated). Probe set intensities were obtained by robust multi-array average background correction, quantile normalization, median polish summarization, and $\log_2$ transformation. Data integrity was then assessed by standard GeneChip® quality control parameters. Duplicate blood samples indicated high data consistency; however, nine genes showing significant differences in expression among duplicate samples were excluded from further analysis (14).

Prior to longitudinal analysis, all probes ($n = 22{,}215$) present on the Human Genome U133A 2.0 arrays were examined to assess levels of expression and identify potential confounding factors. Probes ($n = 9{,}524$) exhibiting low levels of expression, low variance in expression, or associations with technical artifact were removed from further analyses. Using the filtered set of reliably expressed probes ($n = 12{,}691$), we first compared baseline levels of gene expression between all lifestyle participants ($n = 65$) and matched controls ($n = 65$) in the substantial and minimal weight loss groups using two-way ANOVA. We then examined expression changes from baseline to 1 year in these weight loss groups, and separately in the respective matched controls, to determine genes that changed significantly over time in each group. Correction for multiple hypothesis testing was performed by stringent False Discovery Rate (FDR) correction following established methods (16).

Gene Set Enrichment Analysis (GSEA) was conducted in BRB-ArrayTools v4.4.0 using the BioCarta database (http://www.biocarta.com/genes/index.asp). GSEA is a functional class scoring analysis used to identify molecular pathways and transcriptional programs that are differentially expressed across networks of genes but may exhibit only subtle differences at the level of individual genes (17). This approach is more powerful for identifying differential expression compared to the more common over-representation analysis or annotation of gene lists based on individually analyzed genes. Gene sets containing more differentially expressed genes than would be expected by chance were identified using the recommended significance threshold of $P < 0.005$ (18).

Transcript validation by qRT-PCR

In participants experiencing substantial weight loss, eight genes were randomly selected for validation. Total RNA samples (200 ng) from the baseline and 1-year examinations from 27 participants with sufficient RNA remaining for analysis were subjected to qRT-PCR

using TaqMan® Gene Expression Assays (Life Technologies). Target gene expression levels were normalized to GAPDH. Duplicate samples were run for each assay and the mean value was analyzed by the $\Delta\Delta C_T$ method (19). A Pearson correlation coefficient was used to assess the relationship between fold-changes based on qRT-PCR and microarray analysis.

Results

Baseline

The average age of intervention participants (45 women and 44 men) was 60.4 years (range 40.7–85.0) and the average age of controls (36 women and 35 men) was 60.6 years (range 40.6–79.7). Despite the prospective matching strategy, participants and controls differed for some variables at baseline: lifestyle participants were heavier ($P < 0.001$), consumed a higher percentage of carbohydrates ($P = 0.034$), had lower exercise capacity ($P < 0.001$), and higher triglyceride ($P = 0.004$) and leptin ($P = 0.019$) levels (Table 1).

Weight loss and changes in risk factors

Patients experiencing substantial weight loss lost an average of 15.2 ± 3.8% of their total body weight from baseline to 1 year, while those attaining only minimal weight loss lost an average of 3.1 ± 2.5% of body weight (Table 2). The proportion of obese patients at baseline was higher ($P = 0.038$) in the substantial weight loss group (76%), but decreased to 36% by the end of the year, while remaining relatively unchanged (48% at baseline to 45% at 1 year) in the minimal weight loss group. The percentage of patients with diabetes at baseline was similar ($P = 0.775$) between the substantial (21%) and minimal (25%) weight loss groups. Patients losing substantial weight also showed significant improvement in dietary measures, diastolic blood pressure, exercise capacity, triglycerides, insulin, and leptin versus controls. Participants in the minimal weight loss group showed significant changes only for carbohydrate and fat consumption and exercise capacity, but experienced no significant changes in blood pressure, plasma lipids, or inflammatory markers compared to controls (Table 2).

Gene expression

At baseline, no genes showed a significant difference in expression between participants and matched controls using an FDR-corrected P-value of <0.05. Using the MD Anderson Cancer Center sample size calculator (http://bioinformatics.mdanderson.org/MicroarraySampleSize/), with 33 patients in the substantial weight loss group, we had 80% power to detect a ≥1.4-fold-change in gene expression. During 1 year of intensive lifestyle modification, molecular change occurred with successful weight loss—132 unique genes changed significantly in expression (FDR-corrected $P < 0.05$, fold-change ≥1.4) (Supporting Information Table S3). No expression changes were observed in participants who lost minimal weight or in nonintervention controls.

RT-PCR validation

Validation experiments showed a strong positive correlation ($r = 0.964$, $P < 0.0001$) across all eight genes between fold-changes determined by qRT-PCR and microarray analysis (Supporting Information Table S4).

TABLE 1 Dietary measures, cardiovascular risk factors, and plasma biomarkers at baseline in lifestyle modification participants and matched controls

Measure	Controls ($n = 65$)	Participants ($n = 65$)	P-value[a]
Weight (kg)	83.4 ± 15.5	95.9 ± 22.2	<0.001
BMI (kg/m^2)	28.7 ± 4.1	33.6 ± 7.6	<0.001
Dietary measures			
Calories (kcal)	1854 ± 602	2122 ± 858	0.051
% Carbohydrates[b]	50.1 ± 9.9	55.0 ± 11.9	0.034
% Fat[b]	31.2 ± 8.9	27.9 ± 10.7	0.088
Traditional risk factors			
Systolic BP (mm Hg)	136 ± 19	137 ± 17	0.880
Diastolic BP (mm Hg)	79.9 ± 9.1	81.3 ± 10.3	0.380
Exercise capacity (Bruce)	9.8 ± 2.8	6.7 ± 2.4	<0.001
LDL cholesterol (mg/dl)	112 ± 37	112 ± 40	0.978
Total cholesterol (mg/dl)	192 ± 47	192 ± 46	0.870
Triglycerides (mg/dl)	140 ± 85	178 ± 89	0.004
Plasma biomarkers			
C-reactive protein (μg/ml)	2.9 ± 3.8	4.5 ± 5.7	0.068
Insulin (μU/ml)	15.1 ± 7.1	18.1 ± 11.2	0.195
Leptin (ng/ml)	18.5 ± 17.0	24.2 ± 20.3	0.019

BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein.
Data are mean ± SD.
[a]Based on a Wilcoxon nonparametric test.
[b]Percentage of total calories from carbohydrates or fat.

Gene set enrichment analysis

In addition to individual genes, GSEA detected 7 molecular pathways that were significantly down-regulated during successful weight loss. Many of these pathways influence interactions between circulating leukocytes and the vascular endothelium, cellular adhesion, and neutrophil granulation, which are processes important in vascular inflammation (Table 3).

Discussion

Molecular and cellular processes governing inflammation and endothelial activation are known to be important in the pathophysiology of atherosclerotic development. Given the widespread deleterious health effects of the obesity epidemic, identification of therapies that lead to sustainable weight reduction and improvement in vascular dysfunction is critical, yet few studies have examined the beneficial impact of weight loss on molecular pathways that affect endothelial function. In this study, participants in a comprehensive 1-year lifestyle modification program designed to reverse or stabilize progression of CAD showed considerable variability in weight loss, ranging from weight gain of 1.8% to loss of 29.6% of baseline body weight. Substantial weight loss led to improvement in blood pressure, triglycerides, and plasma biomarkers, as well as significant changes in peripheral blood gene expression, while minimal weight loss did not. Molecular pathways governing endothelial activation were significantly down-regulated during successful weight loss. Our observations support the hypothesis that substantial weight loss may be necessary to improve cardiovascular risk

TABLE 2 Change in dietary measures, CVD risk factors, and plasma biomarkers over 1 year in lifestyle participants and matched controls stratified by weight loss success

		Controls		Participants		
Measure	Weight loss group[a]	Baseline	One year % change	Baseline	One year % change	Participants vs. controls, *P*-value[b]
Weight (kg)	High	78.8 ± 14.3[d]	0.0	100.2 ± 19.6[d,e]	−15.2[f]	<0.001
	Low	87.9 ± 15.1	+1.1	91.9 ± 24.3[e]	−3.1[f]	<0.001
BMI (kg/m^2)	High	27.6 ± 3.7[d]	+0.3	34.7 ± 6.6[d]	−15.2[f]	<0.001
	Low	29.9 ± 3.9	+1.3	32.3 ± 8.7	−3.0[f]	<0.001
Dietary measures						
Calories (kcal)	High	1635 ± 548[d]	−2.6	2188 ± 850[d]	−25.3[g]	0.008
	Low	1937 ± 659	−14.3[g]	1937 ± 738	−5.7	0.324
% Carbs[c]	High	50.1 ± 9.0	−3.3	52.7 ± 10.9	+36.8[f]	<0.001
	Low	47.7 ± 10.8[d]	+5.2[g]	56.9 ± 11.6[d]	+24.0[f]	<0.001
% Fat[c]	High	31.1 ± 9.6	+5.8	30.8 ± 10.3[e]	−63.1[f]	<0.001
	Low	34.1 ± 8.8[d]	−5.8	25.8 ± 9.8[d,e]	−54.7[f]	<0.001
Traditional risk factors						
SBP (mm Hg)	High	134 ± 18	−4.3[g]	135 ± 16	−7.3[g]	0.351
	Low	138 ± 19	−8.3[f]	139 ± 17	−7.4[g]	0.775
DBP (mm Hg)	High	77.9 ± 9.2	−1.1	81.2 ± 11.1	−10.2[f]	0.008
	Low	81.7 ± 8.6	−5.1[g]	81.8 ± 9.5	−7.2[g]	0.374
EC (Bruce)	High	9.9 ± 3.0[d]	+0.7	6.8 ± 2.0[d]	+44.0[f]	<0.001
	Low	9.7 ± 2.8[d]	−0.9	6.8 ± 2.4[d]	+28.3[f]	<0.001
LDL (mg/dl)	High	109 ± 38	−2.1	112 ± 40	−0.2	0.792
	Low	115 ± 37	−1.0	112 ± 40	−1.2	0.972
TCH (mg/dl)	High	191 ± 52	−0.7	193 ± 43	−4.3	0.447
	Low	193 ± 42	+0.4	191 ± 50	−2.6	0.469
TG (mg/dl)	High	144 ± 108[d]	+9.8	190 ± 107[d]	−16.9[g]	0.022
	Low	135 ± 52[d]	+11.7	166 ± 65[d]	+3.2	0.602
Plasma biomarkers						
CRP (μg/ml)	High	2.2 ± 1.5[d]	−2.0	4.1 ± 3.5[d]	−32.1[f]	0.071
	Low	3.6 ± 5.1	−15.1	4.8 ± 7.3	−31.5	0.269
Insulin (μU/ml)	High	13.8 ± 6.3[d]	+3.4	21.5 ± 12.4[d,e]	−35.1[f]	<0.001
	Low	16.9 ± 7.9	+4.7	15.0 ± 9.3[e]	−1.5	0.540
Leptin (ng/ml)	High	16.9 ± 12.1[d]	+5.4	24.5 ± 15.2[d]	−50.9[f]	<0.001
	Low	20.3 ± 20.9	+12.1	24.0 ± 24.8	−10.4	0.101

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; TCH, total cholesterol; TG, triglycerides; CRP, C-reactive protein.
Data are mean ± SD.
[a]High, substantial weight loss ($n = 33$); Low, minimal weight loss ($n = 32$).
[b]Based on a matched-pairs t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers) comparing changes from baseline to 1 year in participants versus matched controls.
[c]% Carbs is percentage of total calories from carbohydrates; % Fat is percentage of total calories from fat.
[d]Participants and controls significantly different at baseline ($P<0.05$) based on a Wilcoxon Signed Rank test for matched pairs.
[e]Substantial weight loss and minimal weight loss participants significantly different at baseline ($P<0.05$) based on a Wilcoxon nonparametric test.
[f]$P<0.001$ compared to baseline using a paired t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers).
[g]$P<0.05$ compared to baseline using a paired t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers).

beyond what traditional biomarkers reveal. Improvement in vascular health may require molecular attenuation of interactions between circulating immune cells and the vascular endothelium, which can potentially be achieved with substantial weight loss.

Heightened oxidative stress and elevated levels of circulating inflammatory cytokines are associated with metabolic abnormalities including insulin resistance and diabetes (20). In obese patients, vascular inflammation, impaired endothelial function, and reduced arterial responsiveness (21,22) lead to accelerated rates of atherosclerosis and a higher incidence of major cardiovascular events compared to healthy-weight individuals (23). Although weight loss through diet and/or exercise appears to be the most appropriate therapy to reverse vascular abnormalities associated with obesity (24,25), weight loss

TABLE 3 Molecular pathways differentially expressed over 1 year in lifestyle participants experiencing substantial weight loss

Pathway ID	Pathway name	No. genes	Function	Direction	*P*
h_ahsp	Hemoglobin's chaperone	10	Hemoglobin biosynthesis and stability	Down	0.0002
h_monocyte	Monocyte and its surface molecules	9	Immune/inflammatory response; monocyte interaction with vascular endothelium	Down	0.0014
h_neutrophil	Neutrophil and its surface molecules	7	Immune/inflammatory response; neutrophil interaction with vascular endothelium	Down	0.0021
h_lymphocyte	Adhesion molecules on lymphocyte	7	Immune/inflammatory response; lymphocyte interaction with vascular endothelium	Down	0.0023
h_bArrestin-src	Roles of β-arrestin-dependent recruitment of Src kinases in GPCR signaling	12	Endocytosis; cell proliferation; neutrophil degranulation	Down	0.0023
h_granulocytes	Adhesion and diapedesis of granulocytes	14	Immune/inflammatory response; granulocyte interaction with vascular endothelium	Down	0.0037
h_integrin	Integrin signaling pathway	23	Intracellular signaling; cellular adhesion, mobility, and progression through cell cycle	Down	0.0044

The LS permutation *P*-value for all pathways was < 0.005. GPCR, G protein coupled receptor. Pathways from the BioCarta database available at http://www.biocarta.com/genes/index.asp.

has not been consistently associated with improvements in endothelial function (26), possibly due to differences in the percentage of weight loss achieved by patients across studies. Recent data suggest that the amount of weight reduction may be critical to achieving and maintaining healthy vascular function (27).

Changes in plasma insulin and leptin levels during lifestyle modification corroborated the hypothesis that substantial weight loss may be necessary to produce beneficial anti-inflammatory and anti-oxidative effects on the vasculature. Insulin has been shown to stimulate expression of adhesion molecules (28) and leptin is known to

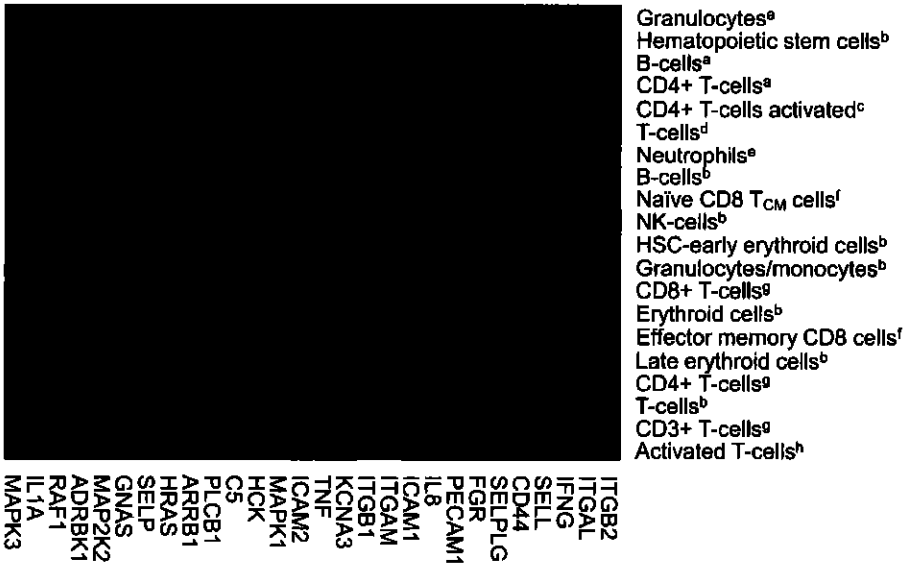

Figure 2 Blue squares denote genes comprising immune and inflammatory pathways, which were significantly down-regulated during substantial weight loss in this study, that are expressed in various subpopulations of human leukocytes. References are provided in Supporting Information Table S6.

promote proliferation and activation of T-lymphocytes, which may contribute to endothelial dysfunction in overweight and obese patients by inducing oxidative stress on endothelial cells (29). In this study, lifestyle participants who lost substantial weight (~15% on average) showed significant reductions in insulin and leptin levels compared to matched controls, but participants losing minimal weight (3% on average) showed no significant changes in circulating markers of inflammation.

Previous research has documented expression changes in genes influencing immune response and vascular inflammation in peripheral blood (30-32) and adipose tissue (33,34) following diet and exercise. Similarly, the genomic response to substantial weight loss during lifestyle change in this study involved down-regulation of genes and pathways associated with endothelial function. Polarization of lymphocytes toward an atherogenic phenotype has been observed in obese patients (35) and changes in the relative abundance of circulating immune cells have been shown to occur in response to long-term weight loss (36). These observations suggest that participants in this study showing substantial weight loss may have experienced shifts in certain leukocyte populations, which may have contributed to changes in peripheral blood gene expression.

Peripheral blood is a complex tissue with diverse cell populations whose relative abundance is dynamic over time. Although whole blood RNA isolation systems such as PAXgene cannot distinguish expression patterns unique to specific subpopulations of circulating cells, they are designed, and have successfully been used, to accurately capture *in vivo* transcription profiles. To address the specificity of our blood-based gene signature approach, we examined expression profiles reported in the literature for major leukocyte subpopulations as well as several control tissues (human brain, liver, pancreas) (37). Only 4 of the 132 genes showing differential expression in peripheral blood during substantial weight loss were elevated in the control tissues, suggesting enrichment for specific subsets of leukocytes during this study. Many genes comprising the differentially expressed BioCarta pathways related to immune and inflammatory responses were expressed in a variety of leukocyte types, but mainly in activated T-cells and other T-cell populations (Figure 2). These specialized cells exhibit different patterns of gene expression that govern their participation in various types of immune responses (38). Activation of T-cells in particular has a major influence on gene expression and is usually associated with production of cytokines and adhesion molecules, which is an important early step in the development of atherosclerosis. Endothelial activation, characterized by adhesion of circulating leukocytes to the vascular endothelium and transmigration across the endothelial barrier, also produces significant changes in gene expression (39). Our data thus suggest that one mechanism through which substantial weight loss may affect vascular health is the down-regulation of molecular pathways associated with endothelial activation and vascular inflammation. Dysregulation of these inflammatory pathways may be attributable to altered transcription within certain leukocyte subpopulations and/or changes in the relative abundance of specialized immune cell types during weight loss.

The prospective, longitudinal nature of this study with validated protocols and matched controls minimized sources of bias and confounding and improved our ability to assess the effects of weight loss on molecular processes relevant to vascular health. The plasma biomarker data strengthened the conclusion that weight loss was an important driver of molecular change. Due to demanding behavioral changes and significant time commitment necessary to successfully complete the intervention, it was impractical to use a randomized study design. We analyzed the data using a per-protocol approach, but included all patients who completed the program whether or not they strictly adhered to the program guidelines. There were no significant differences in the average compliance scores or the percentage of participants meeting compliance targets at the 1-year examination for diet, exercise, and stress management between participants who lost substantial weight and those who lost minimal weight (Supporting Information Table S5); however, adherence data were self-reported by the participants and thus subject to inherent inaccuracies. Because the lifestyle intervention focused on a combination of dietary modification, exercise, and stress reduction, we could not quantify the relative contribution of each modality to overall weight loss and molecular change.

Conclusion

To our knowledge, this is the first study to demonstrate that substantial weight loss during lifestyle modification for improved cardiovascular health is associated with changes in peripheral blood gene expression. Conversely, there were no significant molecular changes associated with minimal weight loss. Weight reduction of at least 10% was associated with significant down-regulation of genetic pathways governing interactions between circulating immune cells and the vascular endothelium. The observed changes in gene expression with substantial weight loss may improve endothelial function and produce meaningful vascular health benefits. As peripheral blood gene expression profiles reflect the pathophysiology of the vasculature, an increased understanding of leukocyte gene expression is necessary to identify mechanisms through which weight loss affects cellular processes involved in cardiovascular risk reduction. Further studies are needed to quantify the effects of weight loss on endothelial function and vascular health. O

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Data in brief

Gene expression profiling during intensive cardiovascular lifestyle modification: Relationships with vascular function and weight loss

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ABSTRACT

Heart disease and related sequelae are a leading cause of death and healthcare expenditure throughout the world. Although many patients opt for surgical interventions, lifestyle modification programs focusing on nutrition and exercise have shown substantial health benefits and are becoming increasing popular. We conducted a year-long lifestyle modification program to mediate cardiovascular risk through traditional risk factors and to investigate how molecular changes, if present, may contribute to long-term risk reduction. Here we describe the lifestyle intervention, including clinical and molecular data collected, and provide details of the experimental methods and quality control parameters for the gene expression data generated from participants and non-intervention controls. Our findings suggest successful and sustained modulation of gene expression through healthy lifestyle changes may have beneficial effects on vascular health that cannot be discerned from traditional risk factor profiles. The data are deposited in the Gene Expression Omnibus, series GSE46097 and GSE66175.

Specifications	
Organism/cell line/tissue	*Homo sapiens*/whole blood
Sex	Male and female
Sequencer or array type	Affymetrix GeneChip HG-U133A 2.0 arrays
Data format	Raw data: CEL/TAR files, Normalized data: SOFT, MINiML, TXT
Experimental factors	Clinical: Standard demographic and clinical information, physiological and biochemical assessment; Molecular: RNA isolated from PAXgene™ tubes, globin reduction treatment of RNA, standard Affymetrix expression analysis, transcript validation by qRT-PCR
Experimental features	Intensive lifestyle modification to stabilize or reverse progression of heart disease over 1 year; participants and retrospectively matched controls with CAD or 2+ risk factors; group comparisons; risk factor correlations with gene expression; functional enrichment and pathways analysis; medication influence
Consent	All patients provided a written informed consent before participation. The study protocol (Pro00009375) was approved by the Chesapeake Institutional Review Board (https://www.chesapeakeirb.com/).
Sample source location	Windber, Pennsylvania, USA

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E-mail address: d.ellsworth@wriwindber.org (D.L. Ellsworth).

Direct link to deposited data

The study is registered as NCT01805492 at ClinicalTrials.gov. Expression data were deposited in the Gene Expression Omnibus (GEO) under series accession numbers GSE46097 and GSE66175 and are available here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46097 and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66175.

Experimental design, materials and methods

Objectives

The main objectives of this project were to 1) characterize longitudinal changes in gene expression in peripheral blood during an intensive cardiovascular lifestyle intervention, and 2) identify associations between gene expression profiles and changes in quantitative heart disease risk factors during the intervention. Our goal was to provide a global view of molecular changes associated with drastic lifestyle modification designed to stabilize or reverse heart disease and ascertain molecular pathways that are important in the development of coronary atherosclerosis.

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Study participants

Inclusion criteria for participation included: 1) adult 21 + years of age, 2) mentally competent to provide informed consent and accurately report adherence, 3) physician diagnosis of coronary artery disease (CAD), which included stable angina, angioplasty, >50% luminal narrowing on coronary angiogram, acute myocardial infarction, bypass surgery, or stent placement, or 2 + CAD risk factors such as obesity (BMI >30), hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), high total cholesterol (>200 mg/dL), diabetes, or family history of heart disease in parents or siblings, 4) approval from personal physician, 5) desire to pursue intensive lifestyle modification as an alternative to, or in conjunction with, standard therapy and motivation to follow the program guidelines for one year, and 6) successful abstinence from smoking for at least three months prior to and during enrollment.

Exclusion criteria were: 1) <21 years of age, 2) presence of unstable coronary syndromes, refractory congestive heart failure, uncontrolled arrhythmia, or high-grade uncorrected cardiac conduction abnormalities, 3) significant left main stenosis (>50%) and ejection fraction <35% in patients who did not have revascularization or were not candidates for revascularization, 4) hypotensive response to exercise, 5) known history of autoimmune disease or systemic/chronic disease requiring chemotherapy or long term treatment, 6) history of substance abuse (including alcohol) without self-certification of abstinence for at least three months, and 7) physical disabilities or medical conditions that would preclude program adherence.

Non-intervention controls were recruited prospectively and matched to program participants based on age (within ±5 years), gender, and disease status (presence of CAD or diabetes mellitus) [1]. Control subjects received only standard care from their primary care physician, did not receive any advice, counseling, or information regarding healthy lifestyle behaviors, and did not participate in any component of the lifestyle intervention.

Intervention

A prospective nonrandomized trial based on the Multicenter Lifestyle Demonstration Project was designed to stabilize or reverse progression of heart disease through comprehensive changes in lifestyle [2]. Participants were recruited by referral from physicians and through advertisements in the media. The lifestyle intervention consisted of a low-fat vegetarian diet (<10% of calories from fat), 180 min/week of moderate aerobic exercise, 1 h of stress management each day, and weekly group support sessions. The year-long program was divided into 2 stages, consisting of an intensive 3-month intervention during which participants were taught to adopt and strictly adhere to the program guidelines followed by a 9-month primarily self-directed maintenance phase.

Clinical information was collected by review of medical records, standard questionnaires, and physical examinations at the baseline, 3-month, and 1-year time points. Demographic and lifestyle factors included the following: age, gender, ethnicity, family history of disease, medication use, various psychometric parameters, and daily caloric intake. Clinical information encompassed: height and weight, systolic and diastolic blood pressure, general endurance, standard lipid panel, lipoprotein profiles, and plasma biomarkers including C-reactive protein, ultra-sensitive insulin, and leptin.

This research was conducted in accordance with the Code of Ethics of the World Medical Association. Participants and controls volunteered to participate in the research study and provided a written informed consent. All research activities were governed by the United States Army Medical Research and Materiel Command (MRMC)/Telemedicine and Advanced Technology Research Center (TATRC) and the Henry M. Jackson Foundation for the Advancement of Military Medicine. Our data reporting followed recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group [3].

Blood collection, RNA isolation, and microarray analysis

Peripheral blood was collected from participants and controls at each time point using the PreAnalytiX PAXgene™ Blood RNA System (Qiagen, Valencia, CA). Blood was placed at room temperature for 4–24 h and frozen at −80 °C. PAXgene™ tubes were thawed overnight at room temperature and RNA isolation was performed using the PAXgene™ blood RNA Kit. Globin mRNA transcripts were depleted from a portion of the total RNA using the GLOBINclear™-Human Kit (Life Technologies, Carlsbad, CA). RNA quality was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and quantity was measured with the Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA). One microgram of globin-depleted RNA was then amplified using the MessageAmp™ II aRNA Amplification System (Life Technologies). Resulting double-stranded cDNA was purified, amplified, and labeled with biotin-11-UTP. Labeled aRNA (15 μg) was subsequently fragmented and hybridized to GeneChip® Human Genome U133A 2.0 arrays (Affymetrix, Santa Clara, CA) and scanned on a GeneChip® Scanner 3000 using standard Affymetrix protocols. All 3 time points for each participant/control were processed together to minimize technical artifact. Further details of RNA isolation and gene expression analysis are available in the Data Supplement of Ellsworth et al. [4].

Quality control analysis

All CEL files (n = 480) were subjected to pre-processing using the Robust Multichip Algorithm (RMA). Probe set intensities were obtained by RMA background correction, quantile normalization, median polish summarization, and $\log_2$ transformation. To assess data integrity, evaluate assay performance, and ensure suitability for analysis, the processed intensity data was subjected to standard GeneChip® quality control parameters: background intensity, raw noise (Q) values, percent present calls, scaling factors, and GAPDH 3′/5′ ratio, and Actin 3′/5′ ratio. In addition, the following QC assessments were conducted: array image analysis to identify artifacts on the array surface, distribution analysis to assess the spread of the data relative to the full probe set, and principal component analysis to summarize overall variance.

Arrays included in the final dataset passed the recommended GeneChip® quality control assessments. The RMA normalized $\log_2$ intensity plot showed consistency of individual arrays relative to the entire dataset (Fig. 1). Principal Component Analysis identified limited variability attributable to laboratory procedures across all arrays (Fig. 2). Comparable percent present values (median = 59.2%, range 48.1–64.8%), assessed using the mean absolute deviation, were observed for all samples (Fig. 3).

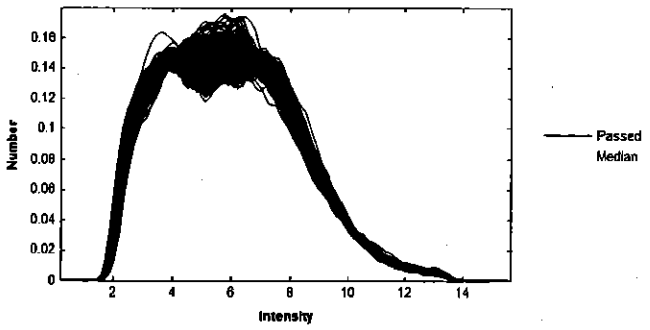

Fig. 1. Intensity graph showing the RMA normalized $\log_2$ intensity for each array. The median intensity curve is highlighted in green.

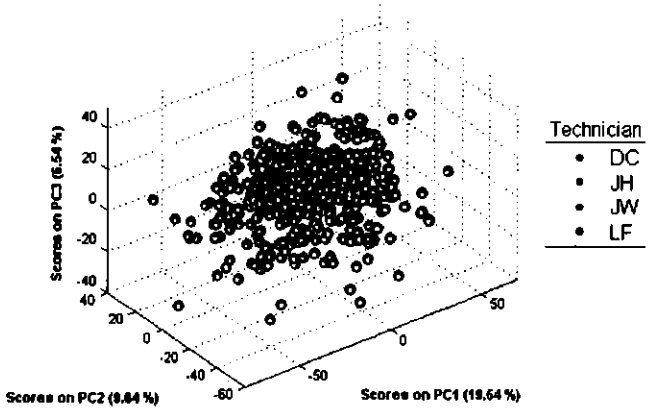

Fig. 2. Three-dimensional scatter plot representing a Principal Component Analysis of all expression arrays colored by laboratory technician.

Duplicate blood samples were collected from 7 randomly-selected participants at each examination and applied to U133A 2.0 arrays as outlined above to evaluate consistency of gene expression among duplicate assays. The average Pearson correlation for the pair-wise comparisons of RMA normalized intensities was 0.992 ± 0.006 (range 0.969–0.996) indicating high repeatability of the microarray data. Paired t-tests identified 9 genes that were differentially expressed between duplicate samples based on a false discovery rate (FDR) adjusted p-value < 0.05 and thus were excluded from further analysis: CKLF-like MARVEL transmembrane domain containing 6 (CMTM6); dehydrogenase/reductase (SDR family) member 9 (DHRS9); guanine nucleotide binding protein (G protein), α11 (GNA11); kelch-like 18 (KLHL18); kinesin family member 1A (KIF1A); mitogen-activated protein kinase 1 interacting protein 1-like (MAPK1IP1L); nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1); transportin 1 (TNPO1); and vesicle-associated membrane protein 1 (synaptobrevin 1) (VAMP1).

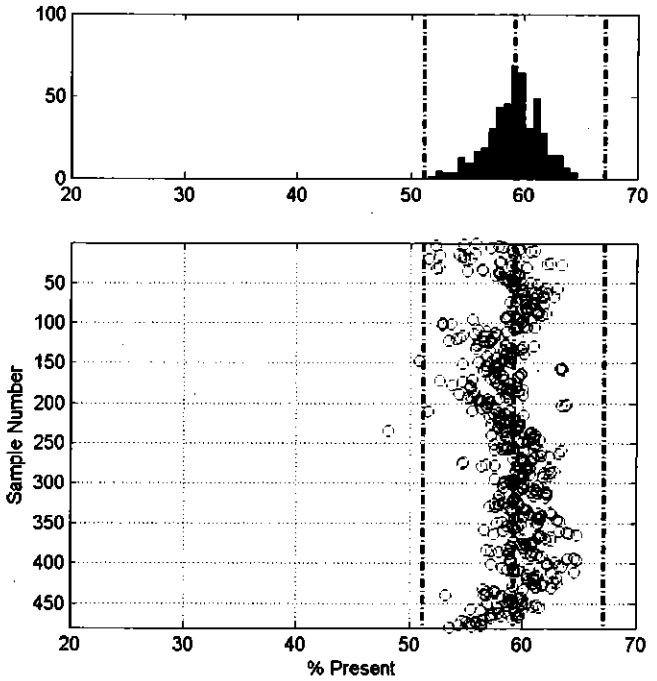

Fig. 3. Histogram (top panel) and scatter plot (bottom panel) showing the percentage of probes on each array yielding detectable expression (percent present calls). The median of the percent present calls is represented by the dashed green line and statistical limits [±3.5 × STD (mean absolute deviation)] by the dashed red lines.

Basic analyses

Our research summarizing changes in physiological [5] and psychosocial risk factors [6], lipoprotein subclass profiles [7], and plasma biomarkers of cardiometabolic risk [8] during the intensive lifestyle intervention has been published previously. In all studies, lifestyle participants experienced dramatic changes in dietary measures and significant improvement in a variety of cardiovascular risk factors compared to controls.

For studies of gene expression, we first selected a subset of 63 participants and 63 matched controls to examine the impact of the lifestyle program on individual gene expression profiles and regulatory pathways important to cardiovascular health. Using ANOVA with FDR correction for multiple testing, we identified 143 genes that were differentially-expressed from baseline to 1 year in lifestyle participants but observed little change in gene expression among controls. Lifestyle modification reduced the expression of proinflammatory genes associated with neutrophil activation and molecular pathways that are important to vascular function [4].

Many genes with the largest fold-changes were significantly correlated with body mass index (BMI) throughout the lifestyle program; therefore, we next examined relationships between weight loss and changes in leukocyte gene expression in 89 lifestyle participants and 71 matched controls. Substantial weight loss (−15.2 ± 3.8%) during the program was associated with improvement in selected cardiovascular risk factors, significant changes in gene expression, and alterations in molecular pathways related to immune function and endothelial activation. Conversely, participants losing minimal weight (−3.1 ± 2.5%) showed only minor changes in risk factors, markers of inflammation, and gene expression compared to non-intervention controls [9].

Discussion

We describe detailed technical and analytical methods for a dataset of 480 Affymetrix GeneChip® U133A 2.0 arrays from 89 participants in an intensive year-long cardiovascular lifestyle intervention and 71 prospectively matched controls. To our knowledge, this is the largest gene expression dataset on participants in a cardiovascular risk reduction program. We believe this data will be of great value to future investigations examining molecular changes that occur in patients embracing healthy lifestyles in addition to the importance of lifestyle in ameliorating cardiovascular risk.

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ORIGINAL ARTICLE

Fatigued on Venus, sleepy on Mars—gender and racial differences in symptoms of sleep apnea

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Abstract
Objective Clinical guidelines for the care of obstructive sleep apnea (OSA) recommend evaluation of daytime sleepiness but do not specify evaluation of fatigue. We studied how subjects with and without OSA experience fatigue and sleepiness, examining the role of gender and race.
Design, setting, patients Consecutive subjects entering our heart health registry completed validated questionnaires including Berlin Questionnaire for OSA, Fatigue Scale, and Epworth Sleepiness Scale. Data analysis was performed only with Whites and Blacks as there were too few subjects of other races for comparison.
Results Of 384 consecutive subjects, including 218 women (57 %), there were 230 Whites (60 %) and 154 Blacks (40 %), with average age of 55.9±12.8 years. Berlin Questionnaires identified 221 subjects (58 %) as having high likelihood for OSA. Fatigue was much more common in women (75 %) than in men (46 %) with OSA ($p<0.001$), while frequency of fatigue was similar in women (30 %) and men (29 %) without OSA ($p=0.86$). In multivariate analysis, men with OSA were sleepier than women; Black men with OSA had higher Epworth scores (mean±SD, 12.8±5.2) compared to White men (10.6±5.3), White women (10.0±4.5), and Black women (10.5±5.2), $p=0.05$. These gender differences were not related to the effects of age, body mass index, perceived stress, sleep duration, or thyroid function.
Conclusions Women report fatigue more commonly with OSA than men. Men experience sleepiness more commonly with OSA than women. The findings suggest that evaluation of sleep disorders must include an assessment of fatigue in addition to sleepiness to capture the experience of women.

Keywords Sleepiness · Fatigue · Obstructive sleep apnea syndrome · Sleep apnea

Abbreviations

BMI	Body mass index
CMS	Centers for Medicare and Medicaid Services
CPAP	Continuous positive airway pressure
EDS	Excessive daytime somnolence
ESS	Epworth sleepiness scale
ICHP	Integrative Cardiac Health Project
IRB	Institutional Review Board
OSA	Obstructive sleep apnea
OSAS	Obstructive sleep apnea syndrome
PSS	Perceived stress scale
SD	Standard deviation
TSH	Thyroid-stimulating hormone

Introduction

Obstructive sleep apnea (OSA) is an important disorder because of its high prevalence [1], the constellation of comorbidities associated with the disorder [2], and the substantial symptoms that OSA may cause [3]. OSA is labeled

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obstructive sleep apnea syndrome (OSAS) when adequate numbers of apneas and hypopneas are accompanied by symptoms such as excessive daytime sleepiness (EDS), fatigue, inattentiveness, moodiness, or morning headaches [4].

In addition to their role in diagnosis of the syndrome, symptoms also serve as important indicators to track response to therapy. A recently published clinical guideline for evaluation and management of OSA [5] endorses the evaluation of sleepiness with the Epworth Sleepiness Scale (ESS) [6] but does not suggest an assessment of fatigue. Other recently published research demonstrates that the ESS is commonly used to evaluate OSA-associated symptoms without incorporation of a scale to measure fatigue [7, 8]. However, subjects with OSA more frequently use terms such as fatigue, tiredness, or lack of energy rather than sleepiness to characterize their symptoms pointing to a lack of connection between the questions asked to elicit symptoms and the experience of symptoms by patients with OSA [9, 10].

Furthermore, symptoms of OSAS are not experienced to the same degree by patients with similar severities of OSA as measured by apnea–hypopnea index or oxygen desaturation [9, 11]. The range and severity of symptoms caused by the sleep disruption of OSA appear to be trait-like qualities for an individual patient [12, 13] and differ markedly among individuals [11]. Substantial data support the contention that sleepiness and fatigue are independent manifestations of sleep disorders and that patients may report one or the other, both or neither while carrying the same objective diagnosis of OSA [9, 10, 14, 15]. While prior research has examined gender differences in symptoms of OSAS [9, 15], we sought to broaden our understanding of the experience of sleepiness and fatigue in subjects with and without OSA with special attention to the roles of gender and race. Such an evaluation has not been previously undertaken.

Methods

This study was conducted in accordance with the amended Declaration of Helsinki and with the approval of the Institutional Review Board (IRB) at the Walter Reed National Military Medical Center in Bethesda, Maryland, which granted approval for the protocol designated #372910. The study design is an analysis of data prospectively collected on consecutive patients enrolled in the Integrative Cardiac Health Project (ICHP) Registry. The ICHP Registry is a cardiovascular disease prevention program operating in a research Center of Excellence for the United States Department of Defense. Because the Registry database could be de-identified before data analysis, an exempt protocol was approved by the IRB (#20012) to perform a secondary analysis on the Registry data and patient consent was not required for the purpose of this analysis.

Patients are self-referred or referred to the ICHP Registry by a health care provider to improve habits of diet, exercise, sleep, and stress management. ICHP is accessible to military health care beneficiaries including active duty service members, retirees, and civilian dependents. The program therefore enrolls a broad spectrum of subjects including a variety of races and ethnic backgrounds, both genders, and a range of ages from 18 to 90 years. The typical patient entering the program is found to have two to four risk factors for cardiovascular disease.

Upon entry, subjects are asked to complete a series of questionnaires (described in detail below) to gather information on demographics, current symptoms, and lifestyle habits. Among the questionnaires are validated surveys to assess sleep behaviors, sleep quality, and daytime symptoms. Data from the questionnaires are reviewed during a medical interview with a nurse practitioner who performs a physical examination with anthropomorphic measures. Patients also submit blood for laboratory tests including a thyroid function panel.

Berlin questionnaire

Of questionnaires available to screen patients for sleep apnea, the Berlin Questionnaire is one of the most commonly utilized and best validated [16]. Permission was granted by the copyright owner to use the questionnaire for this study. As measured by the questionnaire, patients with persistent and frequent signs and symptoms are considered to be at high risk for sleep apnea. Questions about symptoms demonstrated internal consistency (Cronbach correlations, 0.86 to 0.92). With a positive Berlin questionnaire, sleep apnea was predicted with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.89, and a likelihood ratio of 3.79.

Fatigue Scale

The Fatigue Scale is borrowed from the Stanford Patient Education Research Center [17]. The Stanford web site stipulates that the scale is free to use without permission. The Fatigue Scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. The Fatigue Scale was tested on 122 subjects deriving a data set with mean score of 4.89±2.71 points. Subjects who circle 5 to 6 express mild fatigue, 7 to 8 moderate fatigue, and 9 to 10 severe fatigue.

Epworth sleepiness scale

The ESS is the most widely used tool to estimate the subjective symptom of daytime sleepiness [18]. Dr. Johns permits use of the ESS by individual people (including clinicians and researchers) free of charge. Subjects are asked to use a scale of 0 to 3 to estimate their likelihood of dozing in eight different

situations in recent weeks. The individual scores are summed and possible scores range from 0 to 24. Sleepy subjects score 11 or higher and sleepiness can be categorized by scores: 11 to 14, mild sleepiness; 15 to 19, moderate sleepiness; and 20 to 24, severe sleepiness.

Perceived stress scale

The perceived stress scale (PSS) is one of the most widely accepted measures of stress [19]. Dr. Cohen's web site, where a copy of the PSS is provided, states that permission for use of the scale is not necessary when use is for academic research or educational purposes. This validated 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0 to 56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Score in the low 20s reveal moderate stress levels while scores approaching 30 are substantial and concerning.

Statistical analysis

Continuous data that were normally distributed (as determined by the Shapiro–Wilk test) are presented using means with standard deviations (mean±SD): Univariate comparisons are made using the two-sample t test or analysis of variance. Categorical data are presented as counts with proportions and groups are compared using Fisher's exact test. Sleepiness was defined as a score on the ESS of 11 or higher, and fatigue was defined as a score on the Fatigue Scale of 5 or higher.

To adjust for confounding variables, multivariable linear regression was used with either the Fatigue Scale or ESS as the dependent variable and independent variables to include gender, race, age, body mass index (BMI), PSS, thyroid-stimulating hormone (TSH), and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariable models [20]. Data were analyzed using IBM SPSS Statistics for Windows (v. 21.0. IBM Corp. Armonk, NY).

Results

The ICHP Registry enrolled 446 participants. The mean age± standard deviation (SD) of the participants was 55.0± 12.8 years consistent with a spectrum of lifestyles from actively working to semi-retired to fully retired adults. Of the 446 consecutive subjects, 249 women (56 %), there were 234 Whites, 155 Blacks, 13 Hispanics, 2 Asians, and 42 others. Because there were so few participants represented by racial categories other than Whites and Blacks, the other races were not considered further, leaving 389 subjects. Five subjects did not have Epworth or Fatigue Scale data leaving 384 evaluable subjects with an average age of 55.9±12.8 years and including 218 women (57 %).

Fatigue was found in 181 subjects (48 %) and sleepiness in 160 subjects (42 %). The proportion of subjects reporting neither fatigue nor sleepiness, fatigue only, sleepiness only, or both fatigue and sleepiness are shown in Table 1 by race and gender. Women had higher Fatigue Scale scores (Table 2, $p=0.02$), and complained more frequently of fatigue (115 of 215, 53 %) than men (66 of 165, 40 %), while men had significantly higher Epworth scores (Table 3, $p=0.02$), and complained more frequently of sleepiness (77 of 166, 46 %) compared to women (83 of 218, 38 %).

Berlin Questionnaires identified 219 subjects (58 %) as having high likelihood for OSA. There was no difference in thyroid function between subjects with and without a positive Berlin score (mean±SD in each group was 2.2±1.4, $p=0.61$). Symptoms of fatigue and sleepiness are presented in Figs. 1 and 2. Fatigue associated with OSA is more commonly experienced by women than by men, $p<0.001$ (Table 2 and Fig. 1). Sleepiness in association with OSA is more frequently experienced by men, particularly Black men, than by all other categories, $p=0.05$ (Table 3 and Fig. 2).

Univariate analysis of Fatigue Scale scores (Table 2) demonstrates significantly higher scores in younger age groups ($p<0.001$), and in subjects with positive Berlin score ($p<0.001$), higher perceived stress scores ($p<0.001$), and shorter sleep duration ($p<0.001$). Notably, Fatigue Scale scores were not different according to TSH, nor were they different according to BMI categories after factoring in presence of OSA (Table 2).

Univariate analysis of ESS scores (Table 3) show higher scores in younger age categories ($p<0.001$), and in subjects with positive Berlin scores ($p<0.001$), higher perceived stress scores ($p<0.001$), and shorter sleep duration ($p<0.001$). ESS scores were not different according to TSH, nor were they different according to BMI categories after factoring in presence of OSA (Table 3).

To control for confounding demographic and clinical characteristics, multivariable linear regression was used to examine both fatigue and sleepiness. With the Fatigue Scale score as the dependent variable, age and perceived stress score both significantly correlated with fatigue in subjects without OSA. Younger age and higher stress were associated with more fatigue. However, among subjects with OSA, gender was also

Table 1 Symptoms by gender and race

Subject descriptors	All subjects[a] (n=380)	Black women (n=89)	White women (n=126)	Black men (n=63)	White men (n=102)	p value
Age (years)	56.0±12.8	52.9±12.0	56.9±12.0	52.1±13.6	59.9±12.9	<0.001
BMI (kg/m^2)	30.7±5.4	32.5±5.8	29.2±5.3	31.2±4.6	30.7±5.1	<0.001
Not fatigued, not sleepy	141 (37 %)	23 (26 %)	54 (43 %)	25 (40 %)	39 (38 %)	0.007
Fatigued only	81 (21 %)	28 (31 %)	29 (23 %)	6 (9 %)	18 (18 %)	
Sleepy only	58 (15 %)	9 (10 %)	14 (11 %)	15 (24 %)	20 (20 %)	
Both fatigued and sleepy	100 (26 %)	29 (33 %)	29 (23 %)	17 (27 %)	25 (24 %)	

Age, BMI, and the proportion of subjects reporting neither fatigue nor sleepiness, fatigue only, sleepiness only, or both fatigue and sleepiness are shown by race and gender. For age and BMI, comparisons between groups are made using analysis of variance. For the categorical variables of fatigue and sleepiness, comparisons between groups are made using Fisher's exact test. Fatigue was defined as a score on the Fatigue Scale of 5 or higher, and sleepiness was defined as a score on the Epworth Sleepiness Scale of 11 or higher

[a] Three hundred eighty of the 384 subjects had both Epworth and fatigue data

significantly associated with fatigue, with women reporting higher fatigue scores compared to men (Table 4).

Multiple linear regression using ESS score as the dependent variable showed that the independent variable of sleep duration was significantly associated with sleepiness among subjects without OSA, with longer sleep times associated with lower ESS scores. However, among subjects with OSA, PSS and gender were significantly associated with ESS scores. Increases in perceived stress were associated with higher levels of sleepiness. Since female gender was the reference group in the model, the positive beta coefficient for gender indicates a greater degree of sleepiness in men compared to women (Table 5).

Table 2 Fatigue scale data compared for subjects with and without OSA

Fatigue scale		Total			No OSA			OSA		
		n	mean±SD	p value	n	mean±SD	p value	n	mean±SD	p value
All subjects		380	4.4±2.4		161	3.4±2.2		219	5.1±2.3	
Gender	Females	215	4.7±2.5	0.022	105	3.5±2.3	0.58	110	5.8±2.2	<0.001
	Males	165	4.1±2.3		56	3.3±2.1		109	4.5±2.3	
Race	Black	152	4.8±2.4	0.028	56	3.6±2.3	0.54	96	5.4±2.3	0.1
	White	228	4.2±2.4		105	3.4±2.1		123	4.9±2.4	
Gender × race	Black females	89	5.3±2.5	0.002	33	4.0±2.4	0.26	56	6.0±2.3	<0.001
	White females	126	4.2±2.4		72	3.3±2.1		54	5.5±2.2	
	Black males	63	4.0±2.1		23	3.0±1.9		40	4.6±2.0	
	White males	102	4.1±2.4		33	3.6±2.2		69	4.4±2.4	
Age (years)	<50	106	5.6±2.0	<0.001	39	4.6±2.0	<0.001	67	6.2±1.8	<0.001
	50-59	131	4.5±2.5		48	3.6±2.3		83	5.0±2.5	
	60+	143	3.5±2.2		74	2.8±1.9		69	4.3±2.3	
BMI	Normal	51	4.4±2.7	0.02	31	3.5±2.5	0.61	20	5.8±2.6	0.47
	Overweight	129	4.0±2.4		78	3.3±2.2		51	5.0±2.5	
	Obese	200	4.7±2.3		52	3.6±2.1		148	4.3±2.3	
Berlin questionnaire	Normal	161	3.4±2.2	<0.001	161	3.4±2.2				
	OSA	219	5.1±2.4					219	5.1±2.4	
TSH (mU/L)	<4.5	361	4.4±2.4	0.29	154	3.4±2.2	0.3	207	5.1±2.4	0.68
	4.5 +	19	5.0±2.2		7	4.3±1.6		12	5.4±2.4	
PSS (of 56 points)	<21	176	3.4±2.3	<0.001	92	2.7±2.0	<0.001	84	4.1±2.4	<0.001
	21+	200	5.3±2.2		69	4.4±2.1		131	5.8±2.1	
Sleep duration (h)	<6	120	5.4±2.3	<0.001	37	4.3±2.5	0.005	83	5.8±2.1	<0.001
	6+	257	4.0±2.4		122	3.2±2.1		135	4.7±2.4	

Fatigue scale data are presented according to various categories listed on the left column of the table. Comparisons between groups are made using the two-sample t test or analysis of variance

Table 3 Epworth score data compared for subjects with and without OSA

Epworth score		Total			No OSA			OSA		
		n	mean±SD	*p* value	*n*	mean±SD	*p* value	*n*	mean±SD	*p* value
All subjects		384	9.4±5.2		163	7.5±4.7		221	10.9±5.1	
Gender	Females	218	8.9±5.0	0.024	106	7.4±4.8	0.87	112	10.3±4.9	0.096
	Males	166	10.1±5.3		57	7.5±4.4		109	11.4±5.3	
Race	Black	154	10.4±5.4	0.002	57	8.7±5.1	0.015	97	11.5±5.3	0.11
	White	230	8.7±4.9		106	6.8±4.3		124	10.4±4.9	
Gender × race	Black females	91	9.9±5.2	0.001	34	8.9±5.0	0.11	57	10.5±5.2	0.05
	White females	127	8.2±4.8		72	6.7±4.6		55	10.0±4.5	
	Black males	63	11.2±5.6		23	8.4±5.4		40	12.8±5.2	
	White males	103	9.4±5.1		34	7.0±3.5		69	10.6±5.3	
Age (years)	<50	108	11.2±5.5	<0.001	41	8.8±5.3	0.038	67	12.7±5.1	0.002
	50–59	133	9.3±4.9		48	7.8±4.3		85	10.1±5.0	
	60+	143	8.2±4.8		74	6.5±4.3		69	10.0±4.8	
BMI (kg/m^2)	Normal	51	8.7±5.7	0.056	31	6.2±4.7	0.09	20	12.6±5.1	0.26
	Overweight	131	8.8±5.4		79	7.3±4.8		52	11.0±5.5	
	Obese	202	10.0±4.9		53	8.5±4.4		149	10.6±4.9	
Berlin questionnaire	Normal	163	7.5±4.7	<0.001	163	7.5±4.7				
	OSA	221	10.9±5.1					221	10.9±5.1	
TSH (mU/L)	<4.5	365	9.3±5.2	0.19	156	7.3±4.7	0.1	209	10.8±5.2	0.74
	4.5 +	19	10.9±4.3		7	10.3±4.3		12	11.3±4.4	
PSS (of 56 points)	<21	177	8.2±4.7	<0.001	93	7.3±4.6	0.55	84	9.1±4.7	<0.001
	21+	203	10.4±5.3		70	7.7±4.7		133	11.8±5.1	
Sleep duration (h)	<6	121	11.0±5.5	<0.001	37	9.2±5.5	0.023	84	11.7±5.3	0.051
	6+	260	8.7±4.9		124	6.9±4.3		136	10.3±4.9	

Epworth Sleepiness Scale data are presented according to various categories listed on the left column of the table. Comparisons between groups are made using the two-sample *t* test or analysis of variance

Discussion

The salient findings of this study are that symptoms of sleepiness and fatigue experienced in association with OSA have different frequencies by gender and by race even after controlling for confounding variables such as age, BMI, thyroid function, and self-reported total sleep time. In particular, gender was the most strongly predictive variable. These findings are of obvious importance to clinicians evaluating and following subjects with OSA since patients need to be provided with the proper questionnaire tools to quantify their subjective complaints. Evaluating the symptom of fatigue with a

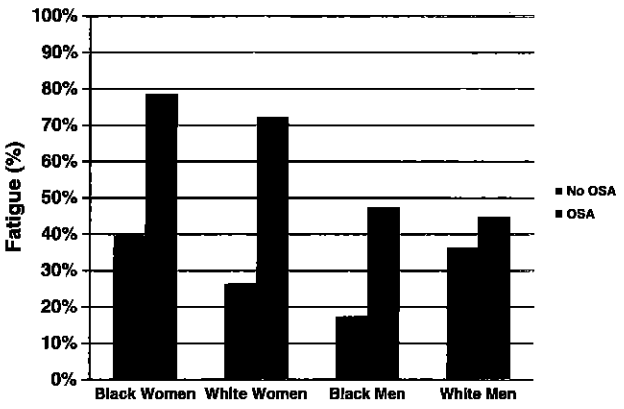

Fig. 1 Frequency of fatigue by race and gender. Fatigue associated with obstructive sleep apnea (OSA) is more commonly experienced by women than by men, $p<0.001$

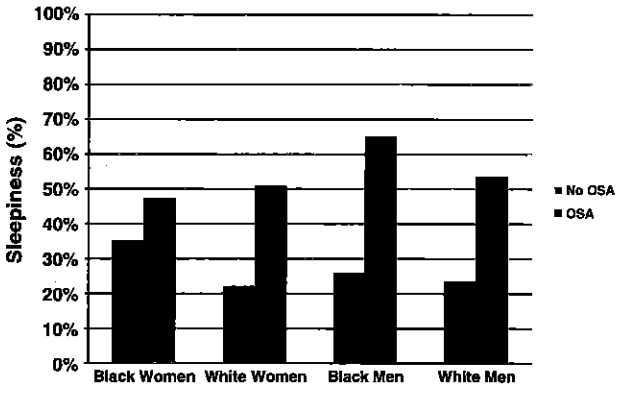

Fig. 2 Frequency of sleepiness by race and gender. Sleepiness in association with obstructive sleep apnea (OSA) is more frequently experienced by men, particularly Black men, than by all other categories, $p=0.05$

Table 4 Results of multivariate linear regression for fatigue score

Independent variables	No OSA		OSA	
	Adjusted coefficients		Adjusted coefficients	
	Beta (95 % CI)	*p* value	Beta (95 % CI)	*p* value
Age	−0.04 (−0.07 to −0.02)	<0.001	−0.03 (−0.05 to −0.004)	0.022
BMI	NS		NS	
PSS	0.09 (0.05 to 0.13)	<0.001	0.09 (0.05 to 0.12)	<0.001
Sleep duration	−0.21 (−0.45 to 0.03)	0.079	−0.14 (−0.36 to 0.09)	0.23
TSH	NS		NS	
Gender[a]	NS		−1.02 (−1.59 to 0.45)	0.001
Race[b]	NS		−0.11 (−0.72 to 0.50)	0.72

To adjust for confounding variables, multivariate linear regression was used with Fatigue Scale as the dependent variable and independent variables to include gender, race, age, BMI, PSS, TSH, and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariate models. NS indicates that a variable was not significant in univariate analysis and was therefore not included in the multivariate model

BMI body mass index, *OSA* obstructive sleep apnea, *PSS* perceived stress scale, *TSH* thyroid-stimulating hormone

[a] Females are reference group

[b] Blacks are reference group

questionnaire designed to quantify sleepiness will not suffice. Likewise, sleepiness cannot be properly evaluated with a questionnaire aimed at the symptom of fatigue. It is of major interest that a sizable proportion of the study subjects (10 to 31 % according to gender and race) experienced fatigue without sleepiness.

The proper documentation of symptoms is also important to gain appropriate allowance by insurance carriers. The National Coverage Determination for continuous positive airway pressure (CPAP) therapy published by the Centers for Medicare and Medicaid Services (CMS) sets the standard for Medicare coverage and is adopted by other insurance providers [21]. CMS considers CPAP therapy reasonable and necessary for patients with a mild category of OSA (apnea hypopnea index or respiratory disturbance index greater than or equal to five events and less than or equal to 14 events per hour) if appropriate symptoms are documented [21]. Without symptoms properly documented in these patients with a mild index of severity, their CPAP therapy would not be justifiable to insurance carriers, including CMS.

Table 5 Results of multivariate linear regression for Epworth sleepiness score

Independent variables	No OSA		OSA	
	Adjusted coefficients		Adjusted coefficients	
	Beta (95 % CI)	*p* value	Beta (95 % CI)	*p* value
Age	−0.04 (−0.09 to 0.01)	0.15	−0.03 (−0.09 to 0.03)	0.28
BMI	0.10 (−0.06 to 0.26)	0.20	NS	
PSS	NS		0.17 (0.08 to 0.25)	<0.001
Sleep duration	−0.71 (−1.27 to −0.16)	0.012	−0.19 (−0.71 to 0.33)	0.47
TSH	0.31 (−0.22 to 0.84)	0.25	NS	
Gender[a]	NS		1.59 (0.27 to 2.90)	0.018
Race[b]	−1.30 (−2.89 to 0.29)	0.11	−0.97 (−2.37 to 0.43)	0.17

To adjust for confounding variables, multivariate linear regression was used with Epworth Sleepiness Scale as the dependent variable and independent variables to include gender, race, age, BMI, PSS, TSH, and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariate models. NS indicates that a variable was not significant in univariate analysis and was therefore not included in the multivariate model

BMI body mass index, *OSA* obstructive sleep apnea, *PSS* perceived stress scale, *TSH* thyroid-stimulating hormone

[a] Females are reference group

[b] Blacks are reference group

The finding of increased sleepiness and fatigue with shorter sleep duration conforms to prior studies that have demonstrated a strong correlation of acute and chronic sleep deprivation with decreased alertness, impaired psychomotor vigilance testing, and shorter sleep latency on mean sleep latency test [22–24]. Likewise, the observation that sleepiness and fatigue decrease with higher age groups agrees with prior research [25, 34]. We speculate that this finding of diminished symptoms with age is further explained by the circumstances that retirement and semi-retirement in older age groups allows for more opportunities to sleep and to sleep on a self-determined schedule.

The association of higher stress levels with increased symptoms of fatigue and sleepiness deserves to be addressed with further scrutiny. Potential explanations are that higher perceived stress levels intensify the experience of other symptoms such as fatigue and sleepiness. It is equally plausible that high stress levels negatively affect sleep latency, sleep continuity, and the restorative quality of sleep. These theoretical considerations warrant further study and suggest that successful stress management may be an intervention as valuable as expansion of sleep time for symptom management.

The findings of a differential experience of symptoms from disturbed sleep according to gender and race are not unique to this study. Recent reports include the observations that women more frequently experience sleep-onset insomnia than men [26] and that White women are more likely to report use of a sleep aid (prescription or nonprescription) [27]. Periodic limb movements of sleep and associated symptoms are much more common in Whites compared to Blacks [28], while estimated prevalence of narcolepsy and its symptoms are higher in women than men and in Blacks than in other racial groups [29]. Blacks are more likely to experience sleep phase advance [30] and both Blacks and women are more likely to report extremes of sleep duration (less than 5 h or greater than 9 h) [31, 32] with attendant elevation in C-reactive protein [33].

In a published review of gender differences, Ye and colleagues raise the concern that differences in symptoms on presentation with OSA may lead to the under-recognition of sleep pathology in women [15]. They note that while the Sleep Heart Health Study [34] did not find the frequency or severity of sleepiness to be affected by gender, the Wisconsin Sleep Cohort Study [1] did report a higher proportion of women with daytime sleepiness than men. Data from the Sleep Heart Health Study analyzed for impact of ethnicity but not gender [35] did find less subjective sleepiness among Blacks than Whites. Other studies report that men tend to report more sleepiness than women [36], and that women prefer to describe their subjective experience of sleep-disordered breathing using terms to denote fatigue, tiredness, and lack of energy [9, 18]. One explanation for these disparate findings regarding the different experiences of symptoms is that the questionnaire instruments may not have allowed participants, especially women, the chance to register symptoms of fatigue.

Research into the differential experience of the subjective symptoms of sleepiness versus fatigue is acknowledged to be difficult [37] and a variety of potential explanations for the disparate published reports above have been advanced. Among the explanations are that men have a less accurate perception of their pathologies than do women, that cultural influences make men less willing to acknowledge symptoms, or that there may be a gender-based neurophysiological explanation for the different experience of OSA [9]. Explanations of racial differences include the impact of socioeconomic conditions [8, 38] and varied subjective interpretation of symptoms due to differing life experiences [39]. However, there are studies that demonstrate clear anatomical differences of the upper airway according to gender and race [40]. Furthermore, a gene association study [41] and gene segregation analysis [42] have documented associations of sleep apnea vulnerability according to race.

A limitation of the current study is that subjects were categorized for the presence of sleep apnea using the Berlin Questionnaire rather than polysomnography. The Berlin Questionnaire is a reasonably sensitive and specific clinical screening tool but it is not the gold standard, suggesting that an appropriate follow-on study may be to repeat our measures in a large population with polysomnography. Another limitation is that races other than Whites and Blacks were not represented in sufficient numbers to include them in this analysis. The symptoms experienced by men and women of other races deserve further discovery.

Another factor potentially limits the ability to generalize our findings to other populations. A third of the subjects in our study sample reported fewer than 6 h of sleep per night. This degree of sleep restriction is higher than that reported in civilian populations and may be a reflection of the military culture from which our study sample derives [43]. A survey of the average sleep duration in the USA reported in 2009 that approximately 40 % of military personnel obtained less than 5 h of sleep per night compared with 8 % in the general population [43].

The data from the current study indicate that the subjective symptoms of sleepiness and fatigue are experienced not just according to gender or race but differentially by both factors simultaneously. These findings underscore the clear need to evaluate patients presenting with sleep disorders using instruments that measure more than just sleepiness and incorporate measures of fatigue and other descriptors commonly voiced by patients suffering from sleep conditions. Clinical centers evaluating patients for sleep disorders would be well advised to incorporate validated instruments for assessing symptoms of fatigue in addition to sleepiness. Future clinical guidelines should incorporate the recommendation that the evaluation of patients with sleep complaints include assessment of symptoms such as fatigue.

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Intensive Cardiovascular Risk Reduction Induces Sustainable Changes in Expression of Genes and Pathways Important to Vascular Function

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Intensive Cardiovascular Risk Reduction Induces Sustainable Changes in Expression of Genes and Pathways Important to Vascular Function

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Background—Healthy lifestyle changes are thought to mediate cardiovascular disease risk through pathways affecting endothelial function and progression of atherosclerosis; however, the extent, persistence, and clinical significance of molecular change during lifestyle modification are not well known. We examined the effect of a rigorous cardiovascular disease risk reduction program on peripheral blood gene expression profiles in 63 participants and 63 matched controls to characterize molecular responses and identify regulatory pathways important to cardiovascular health.

Methods and Results—Dramatic changes in dietary fat intake (−61%; $P<0.001$ versus controls) and physical fitness (+34%; $P<0.001$) led to significant improvements in cardiovascular disease risk factors. Analysis of variance with false discovery rate correction for multiple testing ($P<0.05$) identified 26 genes after 12 weeks and 143 genes after 52 weeks that were differentially expressed from baseline in participants. Controls showed little change in cardiovascular disease risk factors or gene expression. Quantitative reverse transcription polymerase chain reaction validated differential expression for selected transcripts. Lifestyle modification effectively reduced expression of proinflammatory genes associated with neutrophil activation and molecular pathways important to vascular function, including cytokine production, carbohydrate metabolism, and steroid hormones. Prescription medications did not significantly affect changes in gene expression.

Conclusions—Successful and sustained modulation of gene expression through lifestyle changes may have beneficial effects on the vascular system not apparent from traditional risk factors. Healthy lifestyles may restore homeostasis to the leukocyte transcriptome by downregulating lactoferrin and other genes important in the pathogenesis of atherosclerosis.

Clinical Trial Registration—URL: www.clinicaltrials.gov. Unique identifier: NCT01805492

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Key Words: cardiovascular diseases ■ gene expression ■ lifestyle ■ obesity

Cardiovascular disease (CVD) remains the leading cause of death and healthcare burden in the United States, accounting for 1 of every 3 deaths and ≈$313 billion in healthcare-related costs in 2009.[1] Many patients with coronary artery disease (CAD) require expensive surgical interventions, such as coronary artery bypass grafting or percutaneous catheter placement, with significant morbidity and mortality.[2]

Clinical Perspective on p 160

Abundant research has established the relationship between dietary habits and CVD risk,[3,4] and physical activity has been associated with significant reductions in cardiac mortality.[5] Lifestyle modification programs focusing on nutrition and exercise have shown substantial health benefits,[6] in part, by improving endothelial function, reducing cardiovascular events, and slowing or reversing progression of coronary atherosclerosis.[7] Although lifestyle programs are effective in mediating CVD risk through traditional risk factors, little is known about molecular change during intensive lifestyle modification or the significance of molecular responses in long-term CVD risk reduction.

We report the effect of an intensive lifestyle program on peripheral blood gene expression to improve our understanding of cellular and molecular changes that occur during risk reduction in patients with, or at risk for, heart disease. Previous studies have shown that patterns of gene expression in peripheral blood are associated with various CVD phenotypes, including presence of CAD[8] and extent of coronary artery atherosclerosis.[9,10] Our study reveals that gene expression signatures are significantly modulated by rigorous lifestyle behaviors and track with CVD risk profiles over time. These observations suggest that successful and sustained

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modulation of gene expression through lifestyle changes may have beneficial effects on vascular health that cannot be discerned from traditional risk factor profiles.

Methods

Participants

The research protocol was approved by the Institutional Review Board at Windber Medical Center. All subjects volunteered to participate and gave written informed consent. Men and women willing to make comprehensive lifestyle changes completed a prospective, nonrandomized clinical intervention to stabilize or reverse progression of heart disease through changes in lifestyle. Entry criteria were (1) diagnosis of CAD, which included stable angina, angioplasty, evidence of ≥50% luminal narrowing on coronary angiogram, acute myocardial infarction, bypass surgery, or stent placement; or (2) ≥2 CAD risk factors: hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), high total cholesterol (>200 mg/dL), diabetes mellitus, obesity defined as body mass index (BMI) ≥30, or family history of heart disease in parents or siblings. Controls receiving only standard care from their primary physicians were prospectively matched to program participants based on age, sex, and disease status.[11]

Traditional CAD Risk Factors and Diet

Participants were enrolled on an ongoing basis in a lifestyle intervention that consisted of 4 components: (1) low-fat vegetarian diet (<10% of calories from fat), (2) 180 minutes/wk of moderate aerobic exercise, (3) 1 hour of stress management each day, and (4) weekly group support sessions. Demographic and clinical information was obtained by standard questionnaires at baseline, 12 weeks, and 52 weeks. Physiological and biochemical variables were assessed as previously described.[12,13] Dietary data were collected from self-reported 72-hour dietary recall questionnaires. Food Processor v8.4.0 (ESHA Research) was used to determine daily caloric intake and nutrient composition.

Blood Collection, RNA Preparation, and Microarray Analysis

Peripheral blood was obtained from participants at each time point using the PAXgene Blood RNA System (Qiagen). RNA was isolated and quantified following the Qiagen protocol. Globin mRNA transcripts were depleted from a portion of each total RNA sample using the GLOBINclear-Human kit (Ambion). Globin-depleted RNA aliquots (1 μg) were amplified using the MessageAmp II aRNA Amplification System (Ambion). Resulting double-stranded cDNA was purified, amplified, and labeled with biotin-11-uridine-5′-triphosphate. Labeled aRNA (15 μg) was then fragmented and hybridized to GeneChip Human Genome U133A 2.0 arrays (Affymetrix) and scanned on a GeneChip Scanner 3000. Samples were run in batches for globin reduction (n=12), RNA amplification (n=12), and microarray analysis (n=6), keeping all 3 time points for each participant together in the same batch to minimize technical artifact. All gene expression data have been deposited in the Gene Expression Omnibus, series accession number GSE46097 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46097).

Informatics and Analysis

Statistical analysis of CVD risk factors was conducted using JMP (v9.0). Baseline levels for intervention and matched controls were compared using a matched pairs *t* test, and change in risk factors over time was assessed with a Wilcoxon signed-rank test, which analyzed differences in risk factor response among the matched pairs.

Partek Genomics Suite v6.5 (Partek Incorporated) was used to analyze gene expression data from the 378 CEL files, which all passed standard quality control assessment. Duplicate blood samples collected at each time point from 7 random participants indicated high repeatability of the microarray data (average Pearson correlation of normalized intensities was 0.992±0.006; range, 0.969–0.996). Paired *t* tests identified 9 genes that were excluded from further analysis because of significant differences in expression among duplicate samples (Table I in the Data Supplement).

Using 1-way analysis of variance with false discovery rate correction for multiple testing, we first compared baseline levels of gene expression between lifestyle participants and controls and then examined expression changes from baseline to week 12 and baseline to week 52 in lifestyle participants, and separately in controls, to determine genes that changed significantly over time in each group. Stringent gene lists were generated through combined significance (FDR $P<0.05$) and expression change (≥1.1-fold) filtering. Pairwise Pearson product-moment correlations between changes in gene expression and changes in CVD risk factors were calculated using JMP. Functional enrichment analysis was performed on stringent gene lists to identify biological processes controlled by differentially expressed genes. Gene set enrichment analysis, using BRB-ArrayTools v4.2.1 on the Kyoto Encyclopedia of Genes and Genomes database, identified differential expression between groups of genes with common biological function or regulation.[14] To distinguish the effects of the program from the potential influence of medications on gene expression, ancillary analyses were conducted that included only participants who were not taking or did not change the brand or dosage of medications in the following categories: angiotensin-converting enzyme inhibitors, β-blockers, calcium channel blockers, or lipid-lowering drugs.

Transcript Validation by Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA (500 ng) was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and subjected to quantitative reverse transcription polymerase chain reaction using TaqMan Gene Expression Assays (Applied Biosystems). All target gene expression levels were normalized to the housekeeping gene GAPDH. Samples were run in duplicate for each assay, and the mean value was analyzed by the $\Delta\Delta C_T$ method.[15,16] A repeated measures analysis of variance then determined whether fold-change in expression between time points for each gene was statistically significant. Additional materials and methods are described in the Data Supplement.

Results

The average age of lifestyle participants was 60.3 years (range, 44.5–78.4 years). Many participants entered the program with clinically relevant disorders: 41% had hypertension, 60% were clinically obese, and 54% had high cholesterol. At baseline, participants had higher average BMI (32.6±6.7 versus 28.4±3.9) and triglycerides (187±101 versus 133±73 mg/dL) but lower exercise capacity (24.9±7.4 versus 36.7±11.9 mL per kg per minute) than controls ($P<0.01$), despite the prospective matching strategy (Table II in the Data Supplement). Participants who completed the program tended to be older (60.3±9.3 versus 55.3±11.3 years of age) and have higher systolic blood pressure (137±17 versus 131±19 mm Hg) than those who dropped out ($P<0.05$; Table III in the Data Supplement).

Traditional CAD Risk Factors and Diet

The program resulted in substantial reductions in the number of hypertensive (41% down to 17%), obese (60%–37%), and dyslipidemic (54%–37%) patients. In the first 12 weeks, participants showed dramatic improvement in most dietary and CVD risk factors, but little change occurred in controls (Table 1). At 52 weeks, participants maintained significantly lower daily fat intake (−60%; $P<0.001$ compared with matched controls) and higher carbohydrate consumption (+30%; $P<0.001$ versus matched controls). Improvements in BMI (−9%; $P<0.001$), triglycerides (−7%; $P<0.01$), and physical fitness (+38%; $P<0.001$) remained significant compared with matched nonintervention

Table 1. Change in Dietary and Cardiovascular Risk Factors in Participants and Controls

	Controls (n=63)				Participants (n=63)				
Measure	Baseline	12 Weeks	52 Weeks	% Change B-W52*	Baseline	12 Weeks	52 Weeks	% Change B-W52*	Matched Pairs P†
Dietary									
Calories	1750±547	1719±591	1633±462	−6.7	1985±763	1505±293‡	1700±442§	−14.4	0.369
% Carbohydrate‖	49.3±10.0	49.3±7.3	49.9±10.1	+1.2	54.5±10.8	71.2±3.8‡	71.1±3.6‡	+30.4	<0.001
% Fat	32.4±9.3	32.6±6.3	31.7±8.2	−2.2	29.1±10.3	11.2±1.9‡	11.4±3.0‡	−60.7	<0.001
Physiological									
BMI, kg/m²‖	28.4±3.9	28.1±4.1	28.6±4.2	+0.8	32.6±6.7	30.2±6.1‡	29.6±6.2‡	−9.4	<0.001
SBP, mm Hg	134±18	128±15§	126±13‡	−5.7	139±16	124±16‡	129±17‡	−7.6	0.277
DBP, mm Hg	79.3±10.3	77.7±8.6	77.4±8.2	−2.4	82.2±9.9	73.5±8.8‡	76.2±9.2‡	−7.3	0.064
LDL, mg/dL	111±36	107±34	110±36	−1.5	116±42	101±33‡	114±35	−1.3	0.958
TCH, mg/dL	192±46	189±45	190±46	−1.1	200±49	173±42‡	192±43§	−3.9	0.207
TG, mg/dL‖	133±73	151±146	145±77	+8.6	187±101	168±82	174±102	−7.0	0.005
EC (Vo_2 max)‖	36.5±11.8	37.5±11.2	36.4±11.1	−0.1	25.0±8.0	32.0±8.3‡	34.6±10.0‡	+38.4	<0.001

Data presented as mean±SD. There were 36 women and 27 men in each group; 3.7% missing data. BMI indicates body mass index; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCH, total cholesterol; and TG, triglycerides.

*Percent change from baseline to 52 wk.

†From a Wilcoxon signed-rank test for matched pairs comparing changes from baseline to 52 wk in participants and matched controls.

‡$P<0.001$ compared with baseline by a paired t test.

§$P<0.05$ compared with baseline by a paired t test.

‖Baseline values significantly different ($P<0.05$) between participants and controls based on a matched pairs t test.

controls, but systolic blood pressure and lipids showed regression toward pretreatment levels.

Gene Expression

Levels of gene expression were similar between participants and controls at baseline—only 1 (214731_at) of 22277 probes showed a significant difference (FDR $P<0.05$) between groups. Stringent differential analysis identified 26 unique genes (3 upregulated and 23 downregulated) that changed significantly in expression after 3 months of intervention (Table IV in the Data Supplement). By 1 year, 143 characterized genes were significantly upregulated (n=44) or downregulated (n=99) from baseline in lifestyle participants (Table 2; Table V in the Data Supplement). Downregulation of gene expression during lifestyle change occurred far more frequently than expected by chance. Using a binomial distribution calculated as a probability mass function with $P=0.5$, the probability was 3.9×10^{-5} for observing 23 of 26 genes downregulated at 12 weeks and 1.4×10^{-6} for 99 of 143 genes downregulated at 52 weeks. Validation using quantitative reverse transcription polymerase chain reaction confirmed the overall accuracy of the array-based expression results for the transcripts tested (Table 3). In contrast to lifestyle participants, control subjects showed no change in gene expression after 12 weeks (0 genes) and little change by 52 weeks (21 genes; Table VI in the Data Supplement).

Correlations Between CVD Risk Factors and Gene Expression

Throughout the program, many genes exhibiting the largest fold-changes in expression were significantly correlated with BMI (Figure 1). Notably, few genes correlated with blood pressure or plasma lipids after 12 weeks. Dysregulation of several genes was associated with improvement in triglycerides (−10%) during the first 3 months but was not associated after the 12-week examination when triglyceride levels regressed toward baseline.

Functional Analysis

Functional enrichment analysis indicated that genes showing significant changes in expression during the intervention function mainly in immune response and cholesterol storage (Table VII in the Data Supplement). Genes with the greatest changes in expression at 12 weeks showed regression by 52 weeks (Figure 2). Expression of the majority of immune response genes (65%) closely paralleled the substantial improvement followed by regression pattern observed for some traditional risk factors. In contrast, many cholesterol/lipid homeostasis genes (67%) showed a pattern of continual change throughout the program similar to BMI.

Gene set enrichment analysis provided additional insight into molecular pathways regulated by cardiovascular risk factor modification but that were subtle at the individual gene level. Table 4 shows Kyoto Encyclopedia of Genes and Genomes pathways with Efron–Tibshirani max mean statistic[17] ≤0.001 at 12 and 52 weeks. Pathways affected early in lifestyle modification were related to carbohydrate metabolism, glycoprotein hormone levels, and cytokine production, whereas pathways altered later control steroid hormones, cell mobility, and signal transduction and inflammation.

Effects of Medications

Participants were taking 79 different prescription medications at baseline. To determine whether common cardiovascular medications affected gene expression, we examined subgroups of participants based on medication use. In these analyses, changes in expression in participants not taking cardiovascular medications or whose medication levels did not change during

Table 2. Genes Showing Greatest Fold-Change in Expression During CVD Risk Factor Modification

Probe ID	Gene Name	Symbol	Fold-Change	Gene Ontology Biological Process*
202018_s_at†	Lactotransferrin	LTF	−1.67	Immune response, ion transport, iron homeostasis
221748_s_at	Tensin 1‡	TNS1	−1.55	Cell migration, cell-substrate junction assembly
212531_at†	Lipocalin-2	LCN2	−1.47	Transporter activity; binding§
206676_at†	Carcinoembryonic antigen–related CAM8	CEACAM8	−1.44	Immune response
214407_x_at	Glycophorin B (MNS blood group)‡	GYPB	−1.41	Signal transduction; receptor activity§
206698_at	X-linked Kx blood group	XK	−1.41	Amino acid transport
206665_s_at	BCL2-like 1	BCL2L1	−1.39	Response to hypoxia/oxidative stress, apoptosis
203502_at	2,3-bisphosphoglycerate mutase	BPGM	−1.37	Carbohydrate metabolism, glycolysis, respiration
203115_at	Ferrochelatase‡	FECH	−1.35	Cholesterol metabolism, metabolites/energy
207802_at†	Cysteine-rich secretory protein 3	CRISP3	−1.32	Immune response, defense response
208470_s_at†	Haptoglobin/haptoglobin-related protein‡	HP/HPR	−1.30	Defense response, proteolysis, iron homeostasis
212768_s_at†	Olfactomedin 4	OLFM4	−1.29	Cell adhesion, protein binding
213446_s_at	IQ motif containing GTPase-activating protein 1‡	IQGAP1	−1.28	Small GTPase-mediated signal transduction
208632_at	Ring finger protein 10	RNF10	−1.28	Transcription, Schwann cell proliferation
221627_at	Tripartite motif containing 10	TRIM10	−1.28	Erythrocyte differentiation; protein/ion binding§
218418_s_at	KN motif and ankyrin repeat domains 2	KANK2	−1.28	Transcription apoptosis, cell proliferation
217878_s_at	Cell division cycle 27 homolog‡	CDC27	−1.27	Cell proliferation, cell division
210244_at†	Cathelicidin antimicrobial peptide	CAMP	−1.27	Defense response
200615_s_at	Adaptor-related protein complex 2, β1	AP2B1	−1.26	Protein transport, defense response
205557_at†	Bactericidal/permeability-increasing protein	BPI	−1.25	Immune response; lipid binding§
211993_at	WNK lysine–deficient protein kinase 1	WNK1	−1.25	BP regulation, phosphorylation, ion transport

Stringent gene list of changes at 52 wk with combined significance (FDR $P<0.05$) and expression change (≥1.25-fold) filtering. BCL2 indicates B-cell CLL/lymphoma 2; BP, blood pressure; CAM, cell adhesion molecule; and CVD, cardiovascular disease.

*Derived from NetAffx Analysis Center (http://www.affymetrix.com/analysis/index.affx).

†Probes were significant at 12 wk.

‡Three probes for TNS1 and GYPB and 2 probes for FECH, HP/HPR, IQGAP, and CDC27 showed a significant fold-change from baseline to 52 wk.

§Gene Ontology molecular function.

the study were similar to changes in all participants, showing that prescription medication use did not have significant effects on gene expression during lifestyle change (Table 5).

Discussion

Participants who completed a comprehensive lifestyle intervention designed to reverse or stabilize progression of CAD dramatically changed their dietary habits and significantly increased physical activity, which led to substantial weight loss during 1 year. We have previously shown that CVD risk reduction through intensive lifestyle change has positive effects on vascular and mental health by reducing cardiometabolic risk,[12] modulating plasma lipoprotein profiles,[13] and improving clinical measures of depression and stress.[18] Here, we show that

Table 3. Validation of Differential Gene Expression During CVD Risk Factor Modification

	Controls (n=45)				Participants (n=44)				
Gene	12-Week Fold-Change	*P* Value*	52-Week Fold-Change	*P* Value†	12-Week Fold-Change	*P* Value*	52-Week Fold-Change	*P* Value†	Time×CS-CN *P* Value‡
LTF	+0.67	0.469	+0.89	0.328	−2.01	0.002	−1.72	0.026	0.037
LCN2	+0.58	0.202	+0.62	0.187	−0.91	0.078	−0.98	0.057	0.020
CEACAM8	+1.11	0.274	+1.22	0.279	−3.44	0.006	−1.75	0.049	0.010
CRISP3	+1.02	0.230	+1.28	0.242	−2.35	0.005	−1.56	0.004	0.007
HP	+0.15	0.484	−0.07	0.983	−0.96	0.007	−1.18	<0.001	0.008
OLFM4	+1.87	0.102	−0.30	0.625	−6.97	0.011	−4.56	0.071	0.026
CAMP	+0.44	0.230	+0.07	0.562	−1.10	0.007	−1.32	0.033	0.012
BPI	+0.16	0.519	−0.17	0.914	−1.18	0.017	−0.81	0.044	0.040

Validation using quantitative reverse transcription polymerase chain reaction and the $\Delta\Delta C_T$ method was conducted on 45 controls and 44 participants with sufficient RNA remaining for analysis. CVD indicates cardiovascular disease; CN, controls; and CS, cases (or participants).

**P* value comparing 12 wk to baseline using a paired *t* test.

†*P* value comparing 52 wk to baseline using a paired *t* test.

‡Between-group *P* value for time variable using a repeated measures analysis of variance comparing program participants (CS) with controls (CN).

B-W12	BMI −7.5%	SBP −11.1%	DBP −10.7%	LDL −12.9%	TCH −13.4%	TG −10.1%	EC +28.1%
LTF		+0.12	+0.18	+0.23	+0.23	+0.14	−0.09
TNS1	+0.11	+0.01	+0.05	−0.06	−0.13	−0.17	+0.09
LCN2		+0.12	+0.08	+0.09	+0.09	+0.08	−0.01
CEACAM8		+0.06	+0.03			+0.09	−0.03
GYPB	+0.04	−0.04	−0.05	−0.05	−0.10	−0.11	+0.13
XK	+0.04	−0.02	−0.02	−0.03	−0.09	−0.18	+0.17
BCL2L1	−0.04	+0.03	+0.07	−0.06	−0.15		+0.10
BPGM	+0.10	−0.06	+0.01	−0.01	−0.05	−0.11	+0.18
FECH	+0.09	−0.04	+0.01	−0.12	−0.15	−0.12	+0.12
CRISP3		+0.02	+0.06	+0.21			−0.14
HP/HPR		+0.07	+0.24	+0.22	+0.24	+0.01	−0.01
OLFM4		−0.01	+0.05			+0.20	+0.06
IQGAP1	+0.03	+0.02	+0.20	+0.04	−0.02		−0.11
RNF10	+0.07	−0.03	+0.04	−0.09	−0.19		+0.05
TRIM10	+0.05	−0.06	−0.05	−0.06	−0.16		+0.10
KANK2	−0.01	−0.07	−0.01	−0.12	−0.20	−0.19	+0.09
CDC27	+0.05	−0.10	+0.01	−0.10	−0.15		−0.07
CAMP		0.00	+0.04	+0.13	+0.18	+0.17	−0.01
AP2B1	+0.07	−0.09	+0.15	+0.02	−0.09		+0.07
BPI		+0.21	+0.17	+0.10	+0.07	−0.02	+0.03
WNK1	+0.01	−0.03	+0.03	−0.12	−0.19	−0.25	+0.09

W12-52	BMI −2.0%	SBP +4.0%	DBP +3.8%	LDL +13.3%	TCH +11.1%	TG +3.4%	EC +8.0%
LTF	+0.24	+0.12	+0.06	−0.19	−0.11	+0.23	
TNS1		+0.11	+0.03	−0.14	−0.09	+0.11	
LCN2		+0.12	+0.05	−0.23	−0.19	+0.07	
CEACAM8		+0.06	+0.01	−0.18	−0.14	+0.15	−0.26
GYPB		+0.07	+0.03	−0.24	−0.22	0.00	
XK		+0.06	−0.01	−0.22	−0.22	−0.02	
BCL2L1		+0.05	−0.03	−0.07	−0.06	+0.02	
BPGM		+0.03	−0.05	−0.20	−0.18	−0.01	
FECH		+0.09	−0.01	−0.18	−0.18	−0.01	
CRISP3		−0.08	−0.05	−0.22	−0.20	+0.08	−0.18
HP/HPR		−0.05	−0.03			+0.09	−0.18
OLFM4	+0.16	−0.01	0.00	−0.15	−0.13	+0.14	−0.17
IQGAP1	0.00		+0.18	+0.10	+0.11	+0.10	−0.03
RNF10		+0.12	+0.04	−0.16	−0.12	+0.09	
TRIM10		+0.07	−0.02	−0.21	−0.20	+0.02	
KANK2		+0.08	−0.09	−0.12	−0.12	−0.02	
CDC27	+0.13	+0.12	+0.04	−0.03	−0.01	+0.05	
CAMP		+0.02	+0.03			−0.02	
AP2B1		+0.08	−0.05	−0.08	−0.04	+0.15	
BPI		+0.17	+0.08			+0.02	−0.24
WNK1	+0.14	+0.16	0.00	+0.03	+0.07	+0.18	−0.17

B-W52	BMI −9.4%	SBP −7.6%	DBP −7.3%	LDL −1.3%	TCH −3.9%	TG −7.0%	EC +38.4%
LTF		+0.16	+0.08	+0.18		+0.14	
TNS1		+0.13	+0.01	+0.05	+0.08	+0.01	−0.26
LCN2		+0.20	+0.05	+0.15	+0.16	+0.08	
CEACAM8		+0.10	+0.07			+0.06	
GYPB		+0.14	+0.05	0.00	−0.02	−0.04	−0.25
XK		+0.12	+0.09	+0.07	+0.05	−0.11	−0.20
BCL2L1	+0.18	+0.10	−0.12	+0.08	+0.06	−0.06	−0.21
BPGM		+0.11	+0.10	+0.09	+0.07	−0.13	−0.21
FECH		+0.16	+0.01	+0.08	+0.07	−0.09	−0.18
CRISP3		−0.02	+0.12	+0.20	+0.24	+0.12	−0.18
HP/HPR		+0.01	+0.25	+0.14	+0.04	−0.11	−0.10
OLFM4		−0.02	+0.06	+0.15	+0.14	+0.02	−0.18
IQGAP1	+0.20	+0.12	+0.04	+0.02	+0.01	0.00	
RNF10		+0.10	−0.05	+0.02	+0.05	+0.03	
TRIM10		+0.12	0.00	0.00	0.00	−0.10	−0.22
KANK2		+0.09	−0.10	+0.02	+0.02	−0.12	−0.21
CDC27	+0.11	−0.02	−0.11	−0.16	−0.16	−0.01	
CAMP		+0.15	+0.21	+0.17	+0.16	+0.04	−0.10
AP2B1		+0.07	−0.13	+0.04	+0.05	−0.05	
BPI		+0.23	+0.17	+0.24	+0.19	−0.07	−0.18
WNK1	+0.20	+0.08	−0.06	−0.06	−0.03	−0.02	−0.16

Figure 1. Pairwise correlations for changes in cardiovascular disease risk factors and gene expression from baseline to 12 weeks (**top**), week 12 to week 52 (**middle**), and baseline to week 52 (**bottom**) during intensive lifestyle modification. Percentages in column headings represent degree of change for each risk factor during the corresponding time interval. Coefficients highlighted in dark green were significant at $P<0.001$, light green $P<0.05$. Risk factor percent changes are group averages from Table 1; changes in gene expression were calculated as a percent change for each gene at week 12 and week 52 using raw expression data. Stringent gene list of changes at 52 weeks with combined significance (FDR $P<0.05$) and expression change (≥1.25-fold) filtering. BMI indicates body mass index; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCH, total cholesterol; and TG, triglycerides.

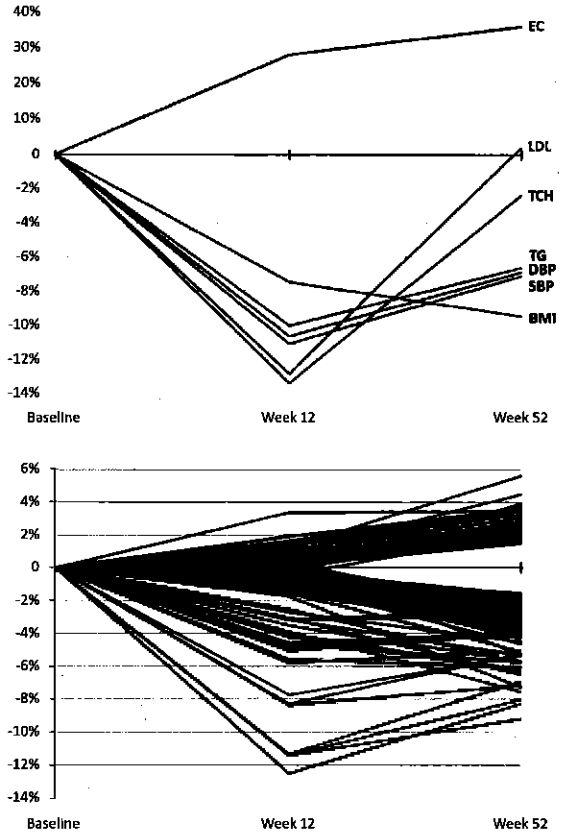

Figure 2. Changes in cardiovascular disease (CVD) risk factors (**top**) and levels of expression for genes differentially regulated during intensive CVD risk reduction (**bottom**). Blue lines, FDR $P<0.05$ and fold-change ≥1.1 but <1.25; red lines, FDR $P<0.05$ and fold-change ≥1.25 at 52 weeks. BMI indicates body mass index; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCH, total cholesterol; and TG, triglycerides.

intensive lifestyle behaviors also modulate gene expression in peripheral blood, suggesting potential CVD risk-reduction mechanisms involving leukocyte function in innate immunity, lipid homeostasis, and inflammation.

Lifestyle modification has been shown to be effective in improving clinically relevant CVD risk factors; however, the extent, persistence, and significance of molecular change accompanying CVD risk reduction are not well known. Daily macronutrients can influence short-term changes in genes related to inflammation, carbohydrate metabolism, and immune function,[19] whereas long-term dietary composition may affect genes and pathways regulating development of atherosclerosis and CVD.[20] Similarly, physical activity induces a variety of rapid biophysical and biochemical responses, including altered expression of genes related to oxidative stress, signal transduction, and inflammation.[21,22] Because expression of diet- and exercise-responsive genes tends to be transient in nature, little is known about the long-term clinical significance of these changes.

During lifestyle modification, participants successfully adopted healthy lifestyle behaviors including a low-fat diet and increased physical activity, which may be important drivers of molecular change. In our analysis of individual

Table 4. KEGG Pathways Differentially Expressed During CVD Risk Factor Modification

ID	Name	No. Genes	Function
Baseline: wk 12			
hsa05120	Epithelial cell signaling in Hpy infection	53	Gene expression and proinflammatory cytokine production in gastric mucosa
hsa04912	GnRH signaling pathway	64	Synthesis/release of gonadotropins; gene expression, stress response
hsa00640	Propanoate metabolism	26	Carboxylic acid metabolism; related to carbohydrate metabolism/glycolysis
Baseline: wk 52			
hsa00150	Androgen and estrogen metabolism	19	Inactivation/catabolism of androgen and estrogen in target tissues
hsa00563	GPI anchor biosynthesis	18	Covalently anchor proteins to cell membranes; signal transduction, inflammation
hsa04810	Regulation of actin cytoskeleton	136	Cellular processes associated with membrane dynamics, cell migration/motility

The Efron–Tibshirani max mean statistic for all pathways was ≤0.001. Available at http://www.genome.jp/kegg/pathway.html. Only galactose metabolism and calcium signaling pathways were differentially expressed in controls at 52 wk. CVD indicates cardiovascular disease; GnRH, gonadotropin-releasing hormone; GPI, glycosylphosphatidylinositol; Hpy, *Helicobacter pylori*; and KEGG, Kyoto Encyclopedia of Genes and Genomes.

genes, immune response and lipid homeostasis were enriched functional categories. The drastic reduction in dietary fat intake during the intervention may influence expression of genes related to lipid storage and transport. Similarly, the predominant downregulation of immune/defense response genes may reflect lower psychological stress and improved vascular health.

Single-gene analysis may miss important effects of lifestyle change on complex molecular pathways; therefore, we conducted gene set enrichment analysis to overview biological processes relevant to CVD risk reduction. Pathways significantly altered were related to physiological changes during the program. The gonadotropin-releasing hormone signaling pathway and the androgen and estrogen metabolism pathway

Table 5. Effects of Medications on Gene Expression From Baseline to 52 Weeks

Probe ID	Symbol	Fold-Change All Participants (n=63)	Fold-Change With Stable or No Lipid Medications (n=51)*	Fold-Change With Stable or No CVD Medications (n=34)†	Among Group *P*‡
202018_s_at	LTF	−1.67	−1.67	−1.70	0.988
221748_s_at	TNS1	−1.55	−1.51	−1.43	0.953
212531_at	LCN2	−1.47	−1.44	−1.48	0.978
206676_at	CEACAM8	−1.44	−1.48	−1.68	0.368
214407_x_at	GYPB	−1.41	−1.34	−1.26	0.768
206698_at	XK	−1.41	−1.43	−1.36	0.933
206665_s_at	BCL2L1	−1.39	−1.35	−1.31	0.946
203502_at	BPGM	−1.37	−1.40	−1.41	0.961
203115_at	FECH	−1.35	−1.31	−1.28	0.933
207802_at	CRISP3	−1.32	−1.32	−1.43	0.637
208470_s_at	HP/HPR	−1.30	−1.31	−1.24	0.856
212768_s_at	OLFM4	−1.29	−1.20	−1.23	0.540
213446_s_at	IQGAP1	−1.28	−1.25	−1.22	0.951
208632_at	RNF10	−1.28	−1.25	−1.18	0.803
221627_at	TRIM10	−1.28	−1.23	−1.21	0.811
218418_s_at	KANK2	−1.28	−1.22	−1.21	0.890
217878_s_at	CDC27	−1.27	−1.26	−1.22	0.961
210244_at	CAMP	−1.27	−1.26	−1.27	0.996
200615_s_at	AP2B1	−1.26	−1.24	−1.22	0.961
205557_at	BPI	−1.25	−1.22	−1.29	0.723
211993_at	WNK1	−1.25	−1.23	−1.17	0.860

CVD indicates cardiovascular disease.

*Includes only participants not taking lipid-lowering medications or whose lipid-lowering medication levels did not change during the study.

†Includes only participants not taking angiotensin-converting enzyme inhibitors, β-blockers, calcium channel blockers, or lipid-lowering medications or whose medication levels for these drugs did not change during the study.

‡Based on a Kruskal–Wallis nonparametric test comparing change in gene expression from baseline to 52 wk among groups.

regulate steroid hormones and activate diverse signaling pathways in nonpituitary tissues that modulate gene expression, cell proliferation, and stress response.[23] Because estrogen and androgen levels are commonly elevated in obesity and weight loss can significantly lower serum estrogen and testosterone levels,[24] weight reduction may lead to changes in pathways affecting sex hormones. Similarly, the propanoate metabolism pathway is related to carbohydrate metabolism and glycolysis; thus, functional changes may reflect increased carbohydrate consumption during the program.

The *Helicobacter pylori* bacterium colonizes the human gastric mucosa and activates multiple signaling pathways.[25] Weight loss through dietary change has been shown to significantly alter the species composition of the intestinal microbiome,[26] thus activation of the *H. pylori* pathway in the first 12 weeks may reflect changes in gut microbiota because of significant dietary changes. Other pathways involving the actin cytoskeleton and glycosylphosphatidylinositol anchor biosynthesis are related to signal transduction, inflammation, and host–pathogen interactions.[27]

Whole blood RNA isolation systems such as PAXgene accurately capture in vivo transcription profiles but cannot distinguish expression signatures unique to specific cell types. To better understand vascular responses to lifestyle modification, we compared genes that were differentially regulated during CVD risk reduction to expression signatures reported for major leukocyte subpopulations. Genes influenced by lifestyle change were expressed in several cell populations, suggesting that different types of circulating cells with unique and specialized functions may be involved in vascular responses to lifestyle modification (Figure 3).

Neutrophils and T-lymphocytes comprise the most abundant leukocyte populations and play essential roles in inflammation and microbial infection. Genes expressed by these specialized cells were downregulated during lifestyle modification, which provides insight into their vascular function and potential role in mediating cardiovascular risk. In particular, neutrophil lactoferrin (LTF; or lactotransferrin) is a multifunctional glycoprotein that serves an important role in host defense and innate immunity. In the circulatory system, LTF

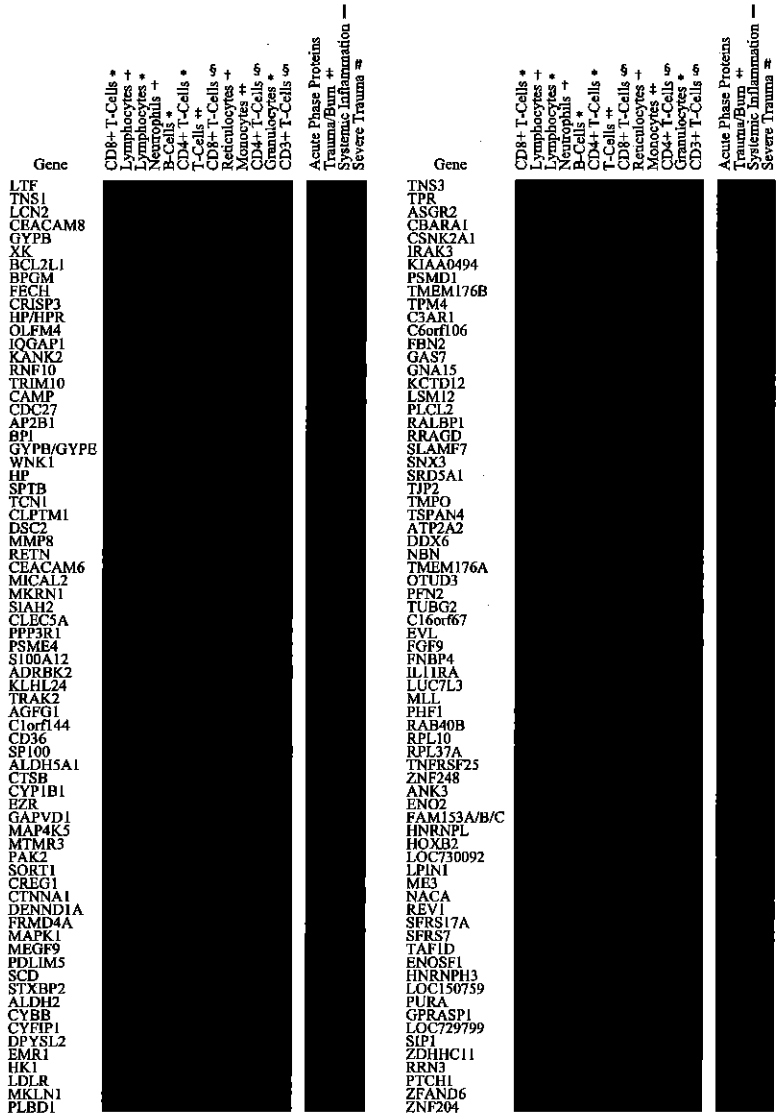

Figure 3. Congruence between cardiovascular disease (CVD) risk-reduction genes and expression signatures reported for major leukocyte subpopulations or CVD-relevant processes. Squares denote whether genes differentially regulated after 52 weeks of intensive lifestyle modification also were expressed (green squares) or not expressed (red squares) in published profiles. *Palmer et al[28]; †Whitney et al[29]; ‡Cobb et al[30]; §Wang et al[31]; ‖Calvano et al.[32]; #Xiao et al.[33]

released by neutrophils regulates production of reactive oxygen species, recruits immune cells to sites of inflammation, and is positively associated with coronary artery stenosis[34] and risk for fatal ischemic heart disease.[35] LTF gene expression is induced in atherosclerotic plaques of human aortas compared with nonatherosclerotic internal thoracic arteries,[36] and salivary LTF concentrations are 60% lower in elite athletes versus sedentary controls.[37] Importantly, in vitro studies have shown that LTF directly affects leukocyte functions that contribute to CVD, including attenuating leukocyte adhesion to vascular endothelial cells, modulating proinflammatory cytokine expression in endothelial cells, and inhibiting processes essential for vascular dysfunction such as proliferation, migration, and angiogenesis.[38] Such parallel evidence implicating LTF in vascular health increases confidence in the validity of our findings and suggests LTF may be therapeutic in patients with CVD who lead unhealthy lifestyles.

Lipocalin-2 (or neutrophil gelatinase-associated lipocalin) is a proinflammatory glycoprotein released by activated neutrophils in response to inflammatory stimuli.[39] Clinical and experimental studies suggest serum lipocalin-2 levels are elevated in obesity and related metabolic complications[40] and positively associated with CAD and cardiac dysfunction.[41,42] Lipocalin-2 is highly expressed in vascular smooth muscle cells and may function in atherosclerotic plaque development by promoting endothelial activation and vascular leukocyte infiltration.[43] Carcinoembryonic antigen–related cell adhesion molecules are immunoglobulin-related glycoproteins that are glycosylphosphatidylinositol-anchored to the surface of granulocytes (neutrophils and eosinophils), where they regulate activation and release of proinflammatory mediators during inflammation and host immunity.[44] Carcinoembryonic antigen–related cell adhesion molecules have been shown to influence neutrophil adhesion to human umbilical vein endothelial cells.[45]

Changes in blood leukocyte gene expression when immune cell function is accentuated, such as systemic inflammation and severe trauma, provide further insight into regulation of leukocyte function during CVD risk reduction. In response to severe bodily injury and infection, leukocytes significantly upregulate expression of numerous genes involved in inflammation and innate immunity.[32,33] Interestingly, genes showing some of the greatest fold increases in expression during severe trauma (LTF, matrix metallopeptidase 8, and haptoglobin) were significantly downregulated during lifestyle change. Lifestyle modification thus may have beneficial effects on vascular health by reducing expression of proinflammatory genes associated with activation of neutrophil granulocytes.

In this study, we controlled for many covariates known to influence blood-based gene expression profiles,[29,46] such as age, sex, time of day, and fasting status, through matching and experimental design. Another complicating factor common among patients with CVD is medication use. Many participants entered the program in poor cardiovascular health, with hypertension, obesity, and hyperlipidemia and, as a result, were taking several prescription medications. These medications may affect cellular function and alter patterns of gene expression in peripheral blood,[47] thus confounding the true effects of lifestyle change. Our analysis indicated that common CVD medications did not have significant effects on peripheral blood gene expression and suggest that alterations in individual genes and multigene pathways were attributable to lifestyle changes.

We showed that intensive lifestyle modification can significantly alter the expression of numerous genes associated with leukocyte function, vascular inflammation, and lipid homeostasis. Fold-changes we observed during a 1-year period in patients undergoing lifestyle modification were comparable in magnitude to differences in expression reported for patients with CVD compared with healthy controls.[8,10] Similar to traditional risk factors, however, these molecular changes seem dynamic, and persistence over time may depend on long-term adherence to healthy behaviors. The number of significantly altered genes increased >5-fold from week 12 to week 52, suggesting that patients who maintain healthy lifestyle behaviors for longer periods of time are likely to experience more diverse molecular change than patients participating in short-term activities. In addition, some conventional risk factors and gene expression profiles showed regression toward baseline after 12 weeks, which corresponded with a lower percentage of participants meeting compliance targets, particularly for exercise and stress management (Table VIII in the Data Supplement). Adherence to cardiovascular treatment regimens involving lifestyle change is particularly difficult, and many patients usually adhere only partially to programmatic goals.[48] Thus, personal motivation and strict adherence are key factors for successful long-term cardiovascular benefit.

Limitations

Intensive lifestyle programs for CVD risk reduction involve demanding behavioral changes that require motivation and a significant time commitment, which likely restrict the applicability of such programs to patients in general. Accordingly, it was impractical to use a randomized study design, which may limit the conclusions that can be drawn from the data, although well-designed case–control studies may be similar to randomized trials for estimating treatment effects.[49] We analyzed the data using a per-protocol (on-treatment) approach but included all patients who completed the program regardless of whether they strictly adhered to the program guidelines. The multifaceted nature of the program precluded us from precisely defining the relative contribution of each component in driving molecular and physiological changes; however, the correlation analysis indicated that many observed changes in gene expression may be attributable to weight loss and physical activity. Furthermore, we could not evaluate long-term changes in gene expression and CVD risk factors beyond 1 year, and we could not assess whether the observed results are achievable outside a controlled clinical environment.

During the intervention, our patients remained under the care of their primary physicians, who may have prescribed medications other than cardiovascular medications. We conducted a subgroup analysis to account for potential effects of common cardiovascular medications on patterns of gene expression, but it is possible that other medications not examined in these analyses influence leukocyte gene transcription.

Peripheral blood is a complex tissue with diverse cell populations whose relative abundance is dynamic over time. Gene

expression studies using whole blood cannot distinguish the effects of cellular demographics from signatures of physiological response. To address this issue, we examined published expression signatures of major leukocyte populations to infer specific cell types involved in response to lifestyle modification; however, rare cell types not examined may play an important role in CVD risk reduction.

Conclusions

CVD prevention through intensive lifestyle changes leads to improvements in clinically relevant cardiac risk factors that may be important in the pathogenesis of atherosclerosis.[50] However, the extent and significance of molecular changes that accompany CVD risk reduction during lifestyle change are poorly understood. There is growing evidence that peripheral blood gene expression reflects the pathophysiology of circulating leukocytes and the vascular endothelium. An increased understanding of dynamic changes in the leukocyte transcriptome during lifestyle modification thus may be crucial for evaluating the efficacy of risk-reduction strategies and understanding mechanisms by which diet and exercise affect cellular processes involved in CVD risk reduction. Conventional risk factors such as low-density lipoprotein cholesterol and blood pressure continue to be primary targets of clinical management for patients with CVD, but as new biochemical and genomic risk factors are identified, it is becoming clear that measures of vascular health go beyond traditional risk factors. A key finding of this study is that successful, sustained modulation and dramatic downregulation of genes, including LTF, through healthy changes in lifestyle may have positive effects on vascular health not readily apparent from traditional risk factors. Future studies are needed to validate changes in gene expression during lifestyle modification and examine the effect of healthy behaviors on leukocyte function and leukocyte–endothelium interactions that are important for cardiovascular health.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Lifestyle interventions designed to reverse or stabilize progression of coronary artery disease successfully ameliorate clinically relevant risk factors important in the pathogenesis of atherosclerosis, but little is known about molecular alterations that accompany lifestyle changes. This study examined the effect of a rigorous cardiovascular risk-reduction program on peripheral blood gene expression profiles to characterize molecular responses and identify regulatory pathways important to cardiovascular health. During intensive lifestyle modification, expression of numerous individual genes and multigene pathways associated with leukocyte function, vascular inflammation, and lipid homeostasis were significantly downregulated. Similar to traditional risk factors, however, changes in the leukocyte transcriptome were dynamic, and persistence over time may depend on long-term adherence to healthy behaviors. As growing evidence suggests that peripheral blood gene expression reflects the pathophysiology of circulating leukocytes and health of the vascular endothelium, successful and sustained modulation of gene expression through changes in lifestyle may have beneficial effects on the vascular system of cardiac patients not apparent from traditional risk factors. Monitoring gene expression is, therefore, potentially useful for determining the vascular benefits of clinical interventions and may identify important targets for drug development.

SUPPLEMENTAL MATERIAL

Participants

The study contained 63 Caucasian participants who completed a prospective, nonrandomized clinical intervention designed to stabilize or reverse progression of CAD through changes in lifestyle. Additional acceptance criteria included physician approval, motivation to follow the program guidelines for one year, and successful abstinence from smoking for at least three months prior to and during enrollment.

Control participants (n=63) receiving only standard care from their primary physicians were matched to lifestyle participants based on gender, age at baseline within five years, and disease status (diagnosis of CAD or risk factors, physician diagnosed diabetes) using a prospective individual matching strategy designed to achieve a balanced distribution of risk factors between intervention and control patients in nonrandomized clinical trials.[1] Control subjects underwent examinations at baseline, 12 weeks, and 52 weeks, but did not receive any advice, counseling, or information regarding healthy lifestyle behaviors and did not participate in any component of the lifestyle change program.

All subjects volunteered to participate in the program and gave written informed consent. Data reporting follows recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group.[2]

Intervention

The lifestyle modification program was divided into two stages over a one-year period. During the first 12-weeks, participants met three times in the first week (16.5 hours total) and

twice a week (10 hours total) during the remaining weeks. During the second phase, participants met once weekly (2 hours) for group support and stress management sessions. The program had four components: 1) a very low fat (<10% of calories from fat) vegetarian diet with emphasis on whole grains, fruits, vegetables, legumes, and soy products; 2) moderate aerobic exercise (180 minutes/week within an individually determined heart rate range) such as walking, rowing, and water aerobics; 3) stress management, which included one hour every day of a combination of yoga poses, relaxation, meditation, imagery, and deep breathing; and 4) group support – two one-hour group sessions per week for the first twelve weeks and one group session of one hour per week thereafter. Participants developed skills for identifying and expressing emotions and for empathetically responding to others.

Patients and controls were enrolled on an ongoing basis in separate cohorts of ~12 individuals per cohort. Clinical staff met with patients twice each week during the first 12 weeks to orient participants to the program and maximize adherence. The remainder of the program was primarily self-directed but included ongoing weekly stress management and group support sessions. All participants completed personal awareness logs each week to summarize daily diet (daily fat, carbohydrate, protein intake calculated as a percentage of calories), exercise (frequency and duration), stress management (frequency and duration), and group support (frequency of meeting attendance). Program staff reviewed compliance forms weekly and provided immediate feedback to participants on progress and adherence.

Traditional CAD Risk Factors

Demographic and clinical information including age, gender, ethnicity, cardiovascular history, and medication use was obtained by standard questionnaires. Height and weight

measurements were used to calculate BMI (kg/m^2). Blood pressure was recorded using a mercury sphygmomanometer on the arm of seated, relaxed subjects. General endurance was determined by a graded treadmill exercise test,[3] which estimated the volume of oxygen each participant could consume (VO_2 max; ml/kg/min) based on exercise intensity and duration using the following formulas:

Men: VO_2 max (ml/kg/min) = $14.8 - (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3)$

Women: VO_2 max (ml/kg/min) = $(4.38 \times T) - 3.9$

T = total time completed during the treadmill test

Fasting blood samples were obtained at each examination and placed directly on ice. Within one hour of collection, plasma aliquots were isolated from whole blood by centrifugation and stored at -80°C. Assays for standard high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, and triglycerides were conducted using the AEROSET™ clinical chemistry system (Abbott Laboratories, Abbott Park, IL).

Blood Collection and RNA Isolation

Peripheral blood samples for gene expression analysis were obtained from participants at each time point using the PAXgene™ Blood RNA System (Qiagen, Venlo, Netherlands). The PAXgene™ tubes were mixed by inverting 10 times immediately after collection. Tubes were incubated at room temperature for 16 hrs following blood collection and then stored at -80°C. Prior to RNA isolation, tubes were removed from -80°C and allowed to thaw at room temperature overnight. RNA was isolated following the Qiagen PAXgene™ blood RNA

recommended protocol with on-column DNase digestion. The PAXgene™ tubes were inverted 10 times after overnight thawing and centrifuged at 4000 rpm for 10 min. After centrifugation, the supernatant was removed and 3 ml of nuclease free water was added to each PAXgene™ tube, which were then vortexed until the pellet was dissolved. The tubes were centrifuged again at 4000 rpm for 10 min and the supernatant was removed leaving the pellet. The pellet was re-suspended in 350 µl of buffer #1 and then transferred to a 1.5 ml microfuge tube. To the microfuge tube, 300 µl of buffer #2 and 40 µl of proteinase K solution were added. The tube was vortexed for 5 sec, then incubated for 10 min at 55°C while shaking at 1400 rpm in a shaker-incubator. After incubation, the lysate was pipetted into a PAXgene™ Shredder spin column and centrifuged for 3 min at maximum speed. The collected supernatant was transferred to a new 1.5 ml microfuge tube, 350 µl of 100% ethanol were added, and the solution was mixed and centrifuged briefly. The sample was then pipetted onto the PAXgene™ RNA spin column and centrifuged for 1 min at maximum speed. The column was washed with 350 µl of buffer #3 by centrifuging the column at maximum speed for 1 min. On-column DNase digestion was performed by pipetting 80 µl of digestion mix onto the filter and incubating at room temperature for 15 min. After the 15 min incubation, the column was washed again with 350 µl of buffer #3. The column was then subjected to two additional wash steps using 500 µl of buffer #4 in each wash. The column was then centrifuged for 1 min at maximum speed to dry and remove any residual ethanol. The isolated RNA was eluted from the column by adding 40 µl of Buffer #5 to the column, incubating at room temperature for 1 min, and centrifuging for 1 min at maximum speed. Following an additional 40 µl elution step, the isolated RNA was denatured by incubation for 5 min at 65°C then placing immediately on ice after incubation. Quality of the resulting RNA samples was assessed on an Agilent® 2100 Bioanalyzer Nano chip (Agilent Technologies, Palo

Alto, CA), and RNA concentrations were determined using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Globin Depletion, RNA Amplification, and Microarray Analysis

To maximize the number of genes detectable in whole-blood RNA,[4] globin mRNA transcripts were depleted from a portion of each total RNA sample (2.5±0.7 µg) using the GLOBINclear™-Human kit (Ambion, Austin, TX). Total RNA was concentrated to a total volume of 14 µl using an Eppendorf Vacufuge. Samples were then incubated at 50°C with 1 µl of Capture Oligonucleotide Mix and 15 µl of 2X Hybridization Buffer. Globin mRNA transcripts were removed by adding 30 µl of prepared Streptavidin Magnetic Beads to the total RNA and incubating at 50°C for 30 min. After incubation, the Streptavidin Magnetic Beads were captured by incubating the tube on a magnetic stand for 5 min. After 5 min, the supernatant containing globin-reduced mRNA was removed and transferred to a new 1.5 ml microfuge tube and placed on ice. To purify the globin-cleared RNA, 100 µl of RNA Binding Buffer and 20 µl of RNA Magnetic Binding Beads were added to the RNA solution. The mixture was vortexed for 10 sec and the RNA Binding Beads were captured by incubating the tube on the magnetic stand for 5 min. The supernatant was removed and discarded and the beads were washed with 200 µl of RNA wash solution, then vortexed for 10 sec. The beads were recaptured by incubation on the magnetic stand for 5 min. The wash solution was removed from the tube and the beads were dried by incubation at room temperature for 5 min. The globin-cleared mRNA was then eluted from the beads by adding 30 µl of 58°C Elution Buffer and incubating at 58°C in a heat block for 5 min. Following incubation, the beads were recaptured on the magnetic stand. The purified globin-cleared mRNA supernatant was then removed and transferred to a new 1.5 ml microfuge

tube. Quality of the globin-depleted RNA samples was determined on the Bioanalyzer and quantity was measured with the NanoDrop® ND-1000.

Globin-depleted RNA aliquots (1 µg) were amplified using the MessageAmp™ II aRNA Amplification System (Ambion) following the manufacturer's recommended procedure. The 1 µg aliquots of globin-depleted RNA were concentrated to a total volume of 8 µl using an Eppendorf Vacufuge. Sample volume was increased to 10 µl by adding 2 µl of Eukaryotic Poly-A RNA (Affymetrix, Santa Clara, CA). Eukaryotic Poly-A RNA, used for quality control during the amplification process to assess target preparation and labelling efficiency, showed the expected linear relationships across all samples according to the default Affymetrix Expression Consol® parameters. The mRNA amplification process started with the creation of the first strand cDNA by reverse transcriptase. The 10 µl samples were first subjected to 10 µl of first strand master mix, which contained RNase inhibitor and ArrayScript reverse transcriptase, then incubated for 2 hrs at 42°C in a thermocycler. Immediately after the 2 hr incubation, 80 µl of second strand master mix (containing DNA polymerase and RNase H) were added to the newly synthesized first strand cDNA and incubated in a thermocycler for 2 hrs at 16°C. The newly synthesized double-stranded cDNA was then purified by adding 250 µl of cDNA binding buffer and pipetting the solution onto a cDNA filter cartridge. The cDNA filter was washed with 500 µl of wash buffer and the purified cDNA was eluted from the filter with 18 µl of preheated nuclease-free water (55°C). The filter was incubated at room temperature for 2 min and then centrifuged at 10,000 x g for 1 min.

Purified double-stranded cDNA was amplified and labeled with biotin-11-UTP (Ambion) during a 14-hr reaction at 37°C by adding 24 µl of IVT master mix containing the T7 enzyme. After the 14 hr incubation, the resulting amplified RNA was purified by adding 350 µl of aRNA

binding buffer and 250 µl of 100% ethanol. The solution was then pipetted onto an aRNA filter cartridge, the cartridge was washed with 650 µl of wash buffer, and the aRNA was eluted from the filter with 100 µl of preheated nuclease-free water (55°C). Quality of the labeled aRNA was assessed on the Bioanalyzer and quantity was measured with the NanoDrop® ND-1000.

Labeled aRNA (15 µg in 24 µl) was fragmented by adding 6 µl of 5X Fragmentation Buffer (Affymetrix). Quality of the fragmented labeled aRNA was assessed on the Bioanalyzer. The fragmented aRNA was then subjected to Eukaryotic Targeted Hybridization by adding a hybridization cocktail that contained 20X GeneChip Eukaryotic Hybridization Controls (Affymetrix). The hybridization cocktail was denatured at 99°C for 5 min, equilibrated to 45°C for 5 min, and then centrifuged at maximum speed for 5 min to pellet all of the insoluble material in the hybridization cocktail. After centrifugation, a 130 µl aliquot of the hybridization cocktail was allowed to hybridize to GeneChip® Human Genome U133A 2.0 arrays (Affymetrix) at 45°C for 16 hrs while being rotated at 60 rpm. Following hybridization, arrays were washed and stained on the GeneChip® Fluidics Station (Affymetrix). The arrays were washed using a non-stringent (6X SSPE, 0.01% Tween-20) and a stringent (100 mM MES, 0.1 M [Na^+], 0.01% Tween-20) wash buffer. The arrays were stained using a SAPE solution (Streptavidin) and antibody solution (Goat IgG and biotinylated antibodies). Finally, the arrays were scanned on a GeneChip® Scanner 3000.

Statistical Analysis

Traditional Risk Factors

Statistical analyses were conducted using JMP® version 9.0; p-values <0.05 were considered statistically significant.

Gene Expression

Data Integrity — CEL files (n=378) from all participants and controls were imported into Partek® Genomics Suite v6.5 (Partek Incorporated, St. Louis, MO). Probe set intensities were obtained by robust multi-array average (RMA) background correction, quantile normalization, median polish summarization, and $\log_2$ transformation. To assess data integrity, the processed intensity data was subjected to standard GeneChip® quality control parameters, which evaluated assay performance and ensured suitability for analysis.

All sample histograms are shown in Data Supplement Figure 1; red arrows indicate profiles from 3 arrays that showed unusual histogram profiles. Normalized intensities from all CEL files were then used in a Principal Component Analysis (PCA) where samples were colored by time point (Data Supplement Figure 2). In the PCA plot, related samples from the same patient (baseline, 12 weeks, 52 weeks) are connected with a solid line. Three arrays (labeled) were clearly distinct; these are the same arrays that showed unusual histogram profiles in Data Supplement Figure 1. The 3 outlier samples identified in the histogram and PCA plot were at the extremes of the recommended ranges for GeneChip® QC parameters. These patients were thus excluded from downstream analysis, and were replaced by patients that met all matching criteria and showed suitable QC parameters. All arrays included in further analyses passed the quality control assessment.

Data Consistency — Duplicate blood samples were collected from seven randomly-selected participants at each time point and applied to U133A 2.0 arrays as outlined above to evaluate the consistency of gene expression among duplicate assays. Overall repeatability of the array data

was first assessed using Pearson correlation coefficients between all pair wise comparisons of RMA normalized intensities. Paired t-tests were then used to identify genes that consistently showed significant differences in expression among the duplicate samples as a filter for exclusion. Comparative analysis identified nine genes that were differentially expressed based on a false discovery rate (FDR) adjusted $P<0.05$ between the duplicate samples and thus were excluded from further analysis.

Differential Expression — Differential gene expression analysis between time points (baseline—12 weeks, baseline—52 weeks) was conducted after RMA background correction, quantile normalization, median polish summarization, and $\log_2$ transformation[5] using ANOVA with participant as the random effects factor and time point as the fixed effects factor. Resulting *P*-values were adjusted by FDR correction for multiple testing.[6] Stringent gene lists were generated through combined significance (FDR-adjusted $P<0.05$) and expression change ($\geq$1.1-fold) filtering. Genes passing the stringent FDR $P<0.05$ criterion were then filtered to select only genes changing in expression by $\geq$1.1 fold. The heat map in Data Supplement Figure 3 provides a visual representation of overall change in gene expression during CVD lifestyle modification relative to non-intervention controls.

Under the assumption that gene expression changes are random, the expectation is that 50% of differentially expressed genes would be down-regulated and 50% up-regulated. We used a binomial distribution as a probability mass function with a probability of 0.5 to calculate the probability of observing 23 of 26 genes down-regulated at 12 weeks and 99 of 143 genes down-regulated at 52 weeks.

Functional enrichment analysis was performed on the stringent gene lists using Gene Ontology (GO) annotations to summarize the most enriched biological processes. The GO annotations were ranked by an enrichment *P*-value, which identified biological processes represented more frequently than expected by chance among genes that changed significantly in expression during the cardiovascular intervention. The enriched GO Biological Process list was then filtered at an enrichment *P*-value ≤ 0.10 and a minimal number of 5 background annotated genes.

Gene expression profiles were compared at the "individual gene" level and "gene set" level. Using predetermined gene sets in the KEGG database, a functional class scoring analysis was conducted with BRB-ArrayTools v4.2.1 and used to identify differential expression between classes.[7] This approach is more powerful for identifying differential expression compared to the more common over-representation analysis or annotation of gene lists based on individually analyzed genes.[8] Gene sets containing more differentially expressed genes than would be expected by chance at a significance threshold of $P \leq 0.001$ were identified.

Transcript Validation by Quantitative Real-Time PCR

Validation qRT-PCR experiments were performed on selected genes to confirm differential expression detected by microarray analysis. Total RNA (500 ng) was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Resulting cDNA (10 ng) was subjected to qRT-PCR using TaqMan® Gene Expression Assays (Applied Biosystems) according to the manufacturer's protocol on an iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). All samples were run in duplicate for each assay and the mean value of the duplicate assays was analyzed by the $\Delta\Delta C_T$ method,[9]

which determined levels of expression for each target gene at each time point. All target gene expression levels were normalized to the housekeeping gene GAPDH. Repeated Measures ANOVA then determined if fold-change in expression from baseline–12 weeks and baseline–52 weeks for each gene was statistically significant.

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Data Supplement Table I. Genes Excluded From Analysis Due to Significant Differences in Expression Among Duplicate Samples

Probe ID	Gene Name	Symbol	Gene Ontology Biological Process*	Fold Difference†
209225_x_at	Transportin 1	TNPO1	Intracellular protein transport	1.26
207976_at	Kelch-like 18 (Drosophila)	KLHL18	Protein binding‡	1.21
203849_s_at	Kinesin family member 1A	KIF1A	Microtubule-based movement; transport	1.15
204248_at	Guanine nucleotide binding protein (G protein), α11 (Gq class)	GNA11	Skeletal development; signal transduction	1.14
213326_at	Vesicle-associated membrane protein 1 (synaptobrevin 1)	VAMP1	Vesicle-mediated transport	1.13
212643_at	Mitogen-activated protein kinase 1 interacting protein 1-like	MAPK1IP1L	—	1.12
211671_s_at	Nuclear receptor subfamily 3 (glucocorticoid receptor)§	NR3C1	Gluconeogenesis; chromatin remodeling	1.12
219799_s_at	Dehydrogenase/reductase (SDR family) member 9	DHRS9	Metabolism; epithelial cell differentiation	1.07
217947_at	CKLF-like MARVEL transmembrane domain containing 6	CMTM6	Chemotaxis	1.06

*Derived from NetAffx™ Analysis Center (http://www.affymetrix.com/analysis/index.affx).
†Based on a paired t-test comparison of Robust Multichip Algorithm (RMA) normalized intensities between 21 duplicate sample pairs using a false discovery rate (FDR) adjusted $P<0.05$.
‡Gene Ontology molecular function.
§Group C, member 1; two probes for NR3C1 showed a significant difference between replicate samples.

Data Supplement Table II. Cardiovascular Risk Factors at Baseline

Measure	n	Controls	Participants	P^*
Dietary components				
Calories (kcal/day)	61	1769 ± 563	2006 ± 753	0.056
% Carbohydrate intake	61	49.4 ± 9.9	54.2 ± 11.0	0.021
% Fat intake	61	32.3 ± 9.2	29.0 ± 10.4	0.092
Physiological measures				
BMI (kg/m^2)	63	28.4 ± 3.9	32.6 ± 6.7	<0.001
Systolic BP (mm Hg)	62	134 ± 18	139 ± 16	0.108
Diastolic BP (mm Hg)	62	79.3 ± 10.3	82.4 ± 9.9	0.072
LDL cholesterol (mg/dl)	59	112 ± 36	116 ± 42	0.573
Total cholesterol (mg/dl)	63	192 ± 46	200 ± 49	0.395
Triglycerides (mg/dl)	63	133 ± 73	187 ± 101	0.002
EC (VO_2 max; ml/kg/min)	52	36.7 ± 11.9	24.9 ± 7.4	<0.001
Age (years)	63	60.3 ± 7.7	60.3 ± 7.8	0.926

Data presented as mean ± SD; BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; EC, exercise capacity.
*Based on a matched-pairs t-test.

Data Supplement Table III. Cardiovascular Risk Factors at Baseline For Drop-outs and Graduates

All Drop-outs vs All Graduates

Measure	n	Drop-outs	n	Graduates	P^*
BMI (kg/m^2)	42	37.4 ± 10.0	107	34.1 ± 7.5	0.107
Systolic BP (mm Hg)	46	131 ± 19	107	137 ± 17	0.046
Diastolic BP (mm Hg)	46	79.9 ± 11.0	107	80.4 ± 9.8	0.675
LDL cholesterol (mg/dl)	43	111 ± 35	106	113 ± 38	0.761
Total cholesterol (mg/dl)	46	193 ± 40	107	194 ± 44	0.948
Triglycerides (mg/dl)	46	198 ± 107	107	182 ± 91	0.382
Exercise capacity (VO_2 max)	44	22.4 ± 8.6	107	23.5 ± 8.4	0.573
Age (years)	46	55.3 ± 11.3	107	60.3 ± 9.3	0.004

Graduates Excluded vs Graduates Included[†]

Measure	n	Excluded	n	Included	P^*
BMI (kg/m^2)	44	36.1 ± 8.0	63	32.6 ± 6.7	0.024
Systolic BP (mm Hg)	44	133 ± 18	63	139 ± 16	0.244
Diastolic BP (mm Hg)	44	77.7 ± 9.1	63	82.2 ± 9.9	0.038
LDL cholesterol (mg/dl)	44	106 ± 30	62	117 ± 42	0.280
Total cholesterol (mg/dl)	44	186 ± 35	63	200 ± 49	0.137
Triglycerides (mg/dl)	44	174 ± 76	63	187 ± 101	0.965
Exercise capacity (VO_2 max)	44	22.3 ± 8.2	63	24.3 ± 8.5	0.159
Age (years)	44	60.4 ± 11.2	63	60.3 ± 7.8	0.795

Data presented as mean ± SD; BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein. Dietary data was unavailable for drop-outs and excluded graduates.
*Based on a Wilcoxon nonparametric test.
†Graduates were excluded from this study if they did not have gene expression data for each time point or did not have a suitable matched control.

Data Supplement Table IV. Genes Showing Differential Expression From Baseline to 12 Weeks in Participants

Probe ID	Gene Name	Symbol	Fold Change	FDR P-value
202018_s_at	lactotransferrin	LTF	-1.86	0.0000
206676_at	carcinoembryonic antigen-related cell adhesion molecule 8	CEACAM8	-1.68	0.0000
212531_at	lipocalin 2	LCN2	-1.55	0.0002
207269_at	defensin, alpha 4, corticostatin	DEFA4	-1.53	0.0012
205033_s_at	defensin, alpha 1; defensin, alpha 1B; defensin, alpha 3, neutrophil-specific	DEFA1; DEFA1B; DEFA3	-1.49	0.0006
212768_s_at	olfactomedin 4	OLFM4	-1.49	0.0002
207802_at	cysteine-rich secretory protein 3	CRISP3	-1.45	0.0000
210244_at	cathelicidin antimicrobial peptide	CAMP	-1.35	0.0001
203757_s_at	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific)*	CEACAM6	-1.33	0.0006
207329_at	matrix metallopeptidase 8 (neutrophil collagenase)	MMP8	-1.30	0.0006
205557_at	bactericidal/permeability-increasing protein	BPI	-1.29	0.0007
208470_s_at	haptoglobin; haptoglobin-related protein	HP; HPR	-1.28	0.0074
206871_at	elastase, neutrophil expressed	ELANE	-1.26	0.0212
205513_at	transcobalamin I (vitamin B12 binding protein, R binder family)	TCN1	-1.25	0.0177
206851_at	ribonuclease, RNase A family, 3 (eosinophil cationic protein)	RNASE3	-1.25	0.0033
220570_at	resistin	RETN	-1.24	0.0000
205653_at	cathepsin G	CTSG	-1.23	0.0074
214575_s_at	azurocidin 1	AZU1	-1.22	0.0387
211657_at	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific)*	CEACAM6	-1.22	0.0074
209771_x_at	CD24 molecule*	CD24	-1.18	0.0074
200832_s_at	stearoyl-CoA desaturase (delta-9-desaturase)	SCD	-1.17	0.0000
216379_x_at	CD24 molecule*	CD24	-1.16	0.0435
202068_s_at	low density lipoprotein receptor	LDLR	-1.15	0.0109
203184_at	fibrillin 2	FBN2	-1.12	0.0143
209616_s_at	carboxylesterase 1 (monocyte/macrophage serine esterase 1)†	CES1	-1.11	0.0282
214321_at	nephroblastoma overexpressed gene	NOV	+1.16	0.0054
203505_at	ATP-binding cassette, sub-family A (ABC1), member 1*	ABCA1	+1.16	0.0036
203504_s_at	ATP-binding cassette, sub-family A (ABC1), member 1*	ABCA1	+1.24	0.0210
203153_at	interferon-induced protein with tetratricopeptide repeats 1	IFIT1	+1.27	0.0402

Stringent gene list with combined significance (FDR $P<0.05$) and expression change ($\geq$1.1-fold) filtering.

*Two probes for ABCA1, CD24, and CEACAM6 showed a significant fold-change from baseline to 12 weeks.

†CES1 (209616_s_at) also showed a significant fold-change from baseline to 52 weeks in controls.

Data Supplement Table V. Genes Showing Differential Expression From Baseline to 52 Weeks in Participants

Probe ID	Gene Name	Symbol	Fold Change	FDR P-value
202018_s_at	lactotransferrin	LTF	-1.67	0.0000
221748_s_at	tensin 1†	TNS1	-1.55	0.0209
212531_at	lipocalin 2	LCN2	-1.47	0.0021
206676_at	carcinoembryonic antigen-related cell adhesion molecule 8	CEACAM8	-1.44	0.0018
214407_x_at	glycophorin B (MNS blood group)†	GYPB	-1.41	0.0446
206698_at	X-linked Kx blood group (McLeod syndrome)	XK	-1.41	0.0392
206665_s_at	BCL2-like 1	BCL2L1	-1.39	0.0457
203502_at	2,3-bisphosphoglycerate mutase	BPGM	-1.37	0.0311
203115_at	ferrochelatase (protoporphyria)*	FECH	-1.35	0.0325
221747_at	tensin 1†	TNS1	-1.33	0.0242
207802_at	cysteine-rich secretory protein 3	CRISP3	-1.32	0.0030
203116_s_at	ferrochelatase (protoporphyria)*	FECH	-1.32	0.0417
207459_x_at	glycophorin B (MNS blood group)†	GYPB	-1.31	0.0487
208470_s_at	haptoglobin; haptoglobin-related protein*	HP; HPR	-1.30	0.0018
212768_s_at	olfactomedin 4	OLFM4	-1.29	0.0320
213446_s_at	IQ motif containing GTPase activating protein 1*	IQGAP1	-1.28	0.0459
208632_at	ring finger protein 10	RNF10	-1.28	0.0483
221627_at	tripartite motif-containing 10	TRIM10	-1.28	0.0230
218418_s_at	KN motif and ankyrin repeat domains 2	KANK2	-1.28	0.0359
217878_s_at	cell division cycle 27 homolog (S. cerevisiae)*	CDC27	-1.27	0.0028
210244_at	cathelicidin antimicrobial peptide	CAMP	-1.27	0.0034
200615_s_at	adaptor-related protein complex 2, beta 1 subunit	AP2B1	-1.26	0.0054
216833_x_at	glycophorin B (MNS blood group)†; glycophorin E	GYPB; GYPE	-1.25	0.0482
205557_at	bactericidal/permeability-increasing protein	BPI	-1.25	0.0038
211993_at	WNK lysine deficient protein kinase 1	WNK1	-1.25	0.0305
206697_s_at	haptoglobin*	HP	-1.24	0.0021
221246_x_at	tensin 1†	TNS1	-1.23	0.0266
208416_s_at	spectrin, beta, erythrocytic	SPTB	-1.22	0.0337
205513_at	transcobalamin I (vitamin B12 binding protein, R binder family)	TCN1	-1.22	0.0253
211136_s_at	cleft lip and palate associated transmembrane protein 1	CLPTM1	-1.21	0.0182
204750_s_at	desmocollin 2	DSC2	-1.21	0.0449
220570_at	resistin	RETN	-1.21	0.0007
207329_at	matrix metallopeptidase 8 (neutrophil collagenase)	MMP8	-1.21	0.0253
203757_s_at	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific)*	CEACAM6	-1.20	0.0431
201285_at	makorin ring finger protein 1	MKRN1	-1.20	0.0316
212473_s_at	microtubule associated monoxygenase, calponin and LIM domain containing 2	MICAL2	-1.20	0.0228
209339_at	seven in absentia homolog 2 (Drosophila)	SIAH2	-1.20	0.0375
219890_at	C-type lectin domain family 5, member A	CLEC5A	-1.19	0.0028
204507_s_at	protein phosphatase 3 (formerly 2B), regulatory subunit B, alpha isoform	PPP3R1	-1.19	0.0285
205863_at	S100 calcium binding protein A12	S100A12	-1.19	0.0493
212220_at	proteasome (prosome, macropain) activator subunit 4	PSME4	-1.19	0.0144
204184_s_at	adrenergic, beta, receptor kinase 2	ADRBK2	-1.18	0.0020

221986_s_at	kelch-like 24 (Drosophila)‡	KLHL24	-1.18	0.0419
202124_s_at	trafficking protein, kinesin binding 2	TRAK2	-1.18	0.0167
211657_at	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific)*	CEACAM6	-1.17	0.0299
213926_s_at	ArfGAP with FG repeats 1	AGFG1	-1.17	0.0007
209555_s_at	CD36 molecule (thrombospondin receptor)*	CD36	-1.16	0.0003
210218_s_at	SP100 nuclear antigen	SP100	-1.16	0.0036
212002_at	chromosome 1 open reading frame 144	C1orf144	-1.16	0.0252
212804_s_at	GTPase activating protein and VPS9 domains 1*	GAPVD1	-1.15	0.0001
211081_s_at	mitogen-activated protein kinase kinase kinase kinase 5	MAP4K5	-1.15	0.0299
203609_s_at	aldehyde dehydrogenase 5 family, member A1	ALDH5A1	-1.15	0.0179
217234_s_at	ezrin‡	EZR	-1.15	0.0325
202435_s_at	cytochrome P450, family 1, subfamily B, polypeptide 1	CYP1B1	-1.15	0.0182
208876_s_at	p21 protein (Cdc42/Rac)-activated kinase 2	PAK2	-1.15	0.0376
200839_s_at	cathepsin B	CTSB	-1.15	0.0338
211507_s_at	myotubularin related protein 3	MTMR3	-1.15	0.0164
212807_s_at	sortilin 1	SORT1	-1.15	0.0051
210840_s_at	IQ motif containing GTPase activating protein 1*	IQGAP1	-1.14	0.0327
208476_s_at	FERM domain containing 4A	FRMD4A	-1.14	0.0139
200832_s_at	stearoyl-CoA desaturase (delta-9-desaturase)	SCD	-1.14	0.0013
201200_at	cellular repressor of E1A-stimulated genes 1	CREG1	-1.14	0.0211
219763_at	DENN/MADD domain containing 1A	DENND1A	-1.14	0.0385
212271_at	mitogen-activated protein kinase 1	MAPK1	-1.14	0.0224
209367_at	syntaxin binding protein 2	STXBP2	-1.14	0.0211
212831_at	multiple EGF-like-domains 9	MEGF9	-1.14	0.0431
200765_x_at	catenin (cadherin-associated protein), alpha 1, 102kDa	CTNNA1	-1.14	0.0415
203242_s_at	PDZ and LIM domain 5	PDLIM5	-1.14	0.0103
214869_x_at	GTPase activating protein and VPS9 domains 1*	GAPVD1	-1.14	0.0018
203922_s_at	cytochrome b-245, beta polypeptide	CYBB	-1.13	0.0028
200762_at	dihydropyrimidinase-like 2	DPYSL2	-1.13	0.0099
207111_at	egf-like module containing, mucin-like, hormone receptor-like 1	EMR1	-1.13	0.0211
202067_s_at	low density lipoprotein receptor*	LDLR	-1.13	0.0026
208923_at	cytoplasmic FMR1 interacting protein 1	CYFIP1	-1.13	0.0093
204423_at	muskelin 1, intracellular mediator containing kelch motifs	MKLN1	-1.13	0.0182
215220_s_at	translocated promoter region (to activated MET oncogene)	TPR	-1.13	0.0247
206488_s_at	CD36 molecule (thrombospondin receptor)*	CD36	-1.13	0.0364
201425_at	aldehyde dehydrogenase 2 family (mitochondrial)	ALDH2	-1.13	0.0099
217853_at	tensin 3	TNS3	-1.13	0.0487
218454_at	phospholipase B domain containing 1	PLBD1	-1.13	0.0211
200697_at	hexokinase 1	HK1	-1.13	0.0305
220034_at	interleukin-1 receptor-associated kinase 3	IRAK3	-1.12	0.0459
202068_s_at	low density lipoprotein receptor*	LDLR	-1.12	0.0238
209344_at	tropomyosin 4	TPM4	-1.12	0.0299
212075_s_at	casein kinase 2, alpha 1 polypeptide	CSNK2A1	-1.12	0.0149
216903_s_at	calcium binding atopy-related autoantigen 1	CBARA1	-1.12	0.0200
220532_s_at	transmembrane protein 176B	TMEM176B	-1.12	0.0194
206130_s_at	asialoglycoprotein receptor 2	ASGR2	-1.12	0.0053
201777_s_at	KIAA0494	KIAA0494	-1.12	0.0085
201198_s_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	PSMD1	-1.12	0.0175
203184_at	fibrillin 2	FBN2	-1.11	0.0161
212188_at	potassium channel tetramerisation domain containing 12	KCTD12	-1.11	0.0028

210959_s_at	steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)	SRD5A1	-1.11	0.0054
219159_s_at	SLAM family member 7	SLAMF7	-1.11	0.0487
216218_s_at	phospholipase C-like 2	PLCL2	-1.11	0.0493
205349_at	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	GNA15	-1.11	0.0144
209264_s_at	tetraspanin 4	TSPAN4	-1.11	0.0299
209754_s_at	thymopoietin	TMPO	-1.11	0.0299
212529_at	LSM12 homolog (S. cerevisiae)	LSM12	-1.11	0.0100
202845_s_at	ralA binding protein 1	RALBP1	-1.11	0.0417
211067_s_at	growth arrest-specific 7	GAS7	-1.11	0.0392
217924_at	chromosome 6 open reading frame 106	C6orf106	-1.11	0.0370
209906_at	complement component 3a receptor 1	C3AR1	-1.11	0.0325
202085_at	tight junction protein 2 (zona occludens 2)	TJP2	-1.11	0.0316
200067_x_at	sorting nexin 3	SNX3	-1.11	0.0492
221524_s_at	Ras-related GTP binding D*	RRAGD	-1.11	0.0233
217881_s_at	cell division cycle 27 homolog (S. cerevisiae)*	CDC27	-1.11	0.0457
204909_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	DDX6	-1.10	0.0167
218345_at	transmembrane protein 176A	TMEM176A	-1.10	0.0236
221523_s_at	Ras-related GTP binding D*	RRAGD	-1.10	0.0498
202905_x_at	nibrin	NBN	-1.10	0.0315
209186_at	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	ATP2A2	-1.10	0.0343
213216_at	OTU domain containing 3	OTUD3	+1.10	0.0182
203894_at	tubulin, gamma 2	TUBG2	+1.10	0.0168
204992_s_at	profilin 2	PFN2	+1.10	0.0228
81811_at	---	---	+1.11	0.0493
217690_at	---	---	+1.11	0.0443
204773_at	interleukin 11 receptor, alpha	IL11RA	+1.11	0.0417
219442_at	chromosome 16 open reading frame 67	C16orf67	+1.11	0.0036
217838_s_at	Enah/Vasp-like	EVL	+1.11	0.0088
204547_at	RAB40B, member RAS oncogene family	RAB40B	+1.11	0.0259
213459_at	ribosomal protein L37a	RPL37A	+1.11	0.0433
206404_at	fibroblast growth factor 9 (glia-activating factor)	FGF9	+1.11	0.0104
202928_s_at	PHD finger protein 1	PHF1	+1.11	0.0236
213269_at	zinc finger protein 248	ZNF248	+1.11	0.0243
208835_s_at	LUC7-like 3 (S. cerevisiae)	LUC7L3	+1.11	0.0274
211841_s_at	tumor necrosis factor receptor superfamily, member 25	TNFRSF25	+1.11	0.0364
220546_at	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	MLL	+1.11	0.0168
212232_at	formin binding protein 4	FNBP4	+1.11	0.0259
221989_at	ribosomal protein L10	RPL10	+1.11	0.0104
213649_at	splicing factor, arginine/serine-rich 7, 35kDa	SFRS7	+1.12	0.0182
212276_at	lipin 1	LPIN1	+1.12	0.0325
218750_at	TATA box binding protein (TBP)-associated factor, RNA polymerase I, D, 41kDa	TAF1D	+1.12	0.0259
205453_at	homeobox B2	HOXB2	+1.12	0.0265
218428_s_at	REV1 homolog (S. cerevisiae)	REV1	+1.12	0.0392
201313_at	enolase 2 (gamma, neuronal)	ENO2	+1.12	0.0049
221860_at	heterogeneous nuclear ribonucleoprotein L	HNRNPL	+1.12	0.0088
222018_at	nascent polypeptide-associated complex alpha subunit	NACA	+1.12	0.0002
204663_at	malic enzyme 3, NADP(+)-dependent, mitochondrial	ME3	+1.12	0.0052

206385_s_at	ankyrin 3, node of Ranvier (ankyrin G)	ANK3	+1.12	0.0451
215211_at	RRN3 RNA polymerase I transcription factor homolog (S. cerevisiae) pseudogene	LOC730092	+1.12	0.0286
214945_at	family with sequence similarity 153, member A; member B; member C	FAM153A; FAM153B; FAM153C	+1.12	0.0267
210269_s_at	splicing factor, arginine/serine-rich 17A	SFRS17A	+1.12	0.0392
213158_at	---	---	+1.13	0.0376
220882_at	---	---	+1.13	0.0034
204020_at	purine-rich element binding protein A	PURA	+1.13	0.0228
213645_at	enolase superfamily member 1	ENOSF1	+1.13	0.0331
210110_x_at	heterogeneous nuclear ribonucleoprotein H3 (2H9)	HNRNPH3	+1.13	0.0315
213703_at	hypothetical protein LOC150759	LOC150759	+1.13	0.0434
221646_s_at	zinc finger, DHHC-type containing 11	ZDHHC11	+1.14	0.0269
217667_at	SEC14-like 1 pseudogene	LOC729799	+1.14	0.0376
204793_at	G protein-coupled receptor associated sorting protein 1	GPRASP1	+1.14	0.0393
211114_x_at	survival of motor neuron protein interacting protein 1	SIP1	+1.14	0.0422
216902_s_at	RRN3 RNA polymerase I transcription factor homolog (S. cerevisiae) pseudogene	LOC653390; LOC730092; RRN3	+1.15	0.0167
220399_at	non-protein coding RNA 115	NCRNA00115	+1.15	0.0199
204140_at	tyrosylprotein sulfotransferase 1	TPST1	+1.15	0.0457
214747_at	zinc finger, BED-type containing 4	ZBED4	+1.16	0.0036
209815_at	patched homolog 1 (Drosophila)	PTCH1	+1.16	0.0305
221973_at	---	---	+1.17	0.0051
222186_at	zinc finger, AN1-type domain 6	ZFAND6	+1.18	0.0168
214823_at	zinc finger protein 204 pseudogene	ZNF204	+1.19	0.0457
216050_at	---	---	+1.48	0.0347

Stringent gene list with combined significance (FDR $P<0.05$) and expression change ($\geq$1.1-fold) filtering; probes in bold also were significant at 12 weeks. Gene information was unknown for 6 probes.

*Two probes for CD36, CDC27, CEACAM6, FECH, GAPVD1, HP, IQGAP1, LDLR, and RRAGD showed a significant fold-change from baseline to 52 weeks.

†Three probes for GYPB and TNS1 showed a significant fold-change from baseline to 52 weeks.

‡Different probes for EZR (208621_s_at) and KLHL24 (221985_at) also showed a significant fold-change from baseline to 52 weeks in controls.

Data Supplement Table VI. Genes Showing Differential Expression From Baseline to 52 Weeks in Controls

Probe ID	Gene Name	Symbol	Fold Change	FDR P-value
219608_s_at	F-box protein 38	FBXO38	-1.19	0.0371
203056_s_at	PR domain containing 2, with ZNF domain	PRDM2	-1.19	0.0371
208621_s_at	ezrin	EZR	-1.19	0.0371
220946_s_at	SET domain containing 2	SETD2	-1.17	0.0329
201299_s_at	MOB1, Mps One Binder kinase activator-like 1B (yeast)	MOBKL1B	-1.15	0.0329
202601_s_at	HIV-1 Tat specific factor 1	HTATSF1	-1.15	0.0413
206521_s_at	general transcription factor IIA, 1, 19/37kDa	GTF2A1	-1.14	0.0371
206500_s_at	chromosome 14 open reading frame 106	C14orf106	-1.14	0.0329
213286_at	zinc finger RNA binding protein*	ZFR	-1.14	0.0371
211949_s_at	nucleolar and coiled-body phosphoprotein 1	NOLC1	-1.14	0.0329
214500_at	H2A histone family, member Y	H2AFY	-1.14	0.0371
209055_s_at	CDC5 cell division cycle 5-like (S. pombe)	CDC5L	-1.13	0.0329
204047_s_at	phosphatase and actin regulator 2	PHACTR2	-1.13	0.0330
33148_at	zinc finger RNA binding protein*	ZFR	-1.12	0.0329
209616_s_at	carboxylesterase 1 (monocyte/macrophage serine esterase 1)	CES1	-1.12	0.0489
202663_at	WAS/WASL interacting protein family, member 1	WIPF1	-1.12	0.0434
210251_s_at	RUN and FYVE domain containing 3	RUFY3	-1.12	0.0489
221985_at	kelch-like 24 (Drosophila)	KLHL24	-1.11	0.0329
206544_x_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin	SMARCA2	-1.11	0.0371
218554_s_at	ash1 (absent, small, or homeotic)-like (Drosophila)	ASH1L	-1.10	0.0413
216652_s_at	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	DR1	-1.10	0.0329
202170_s_at	aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	AASDHPPT	-1.10	0.0457
217570_x_at	---	---	+1.11	0.0380

Stringent gene list with combined significance (FDR $P<0.05$) and expression change ($\geq$1.1-fold) filtering.

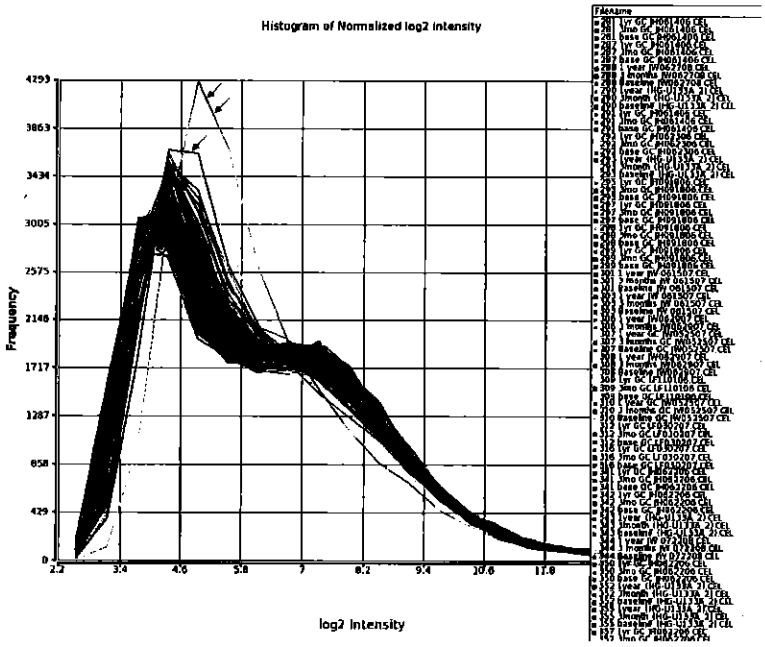
*Two probes for ZFR showed a significant fold-change from baseline to 52 weeks.

Data Supplement Table VII. Functional Enrichment Analysis in Participants

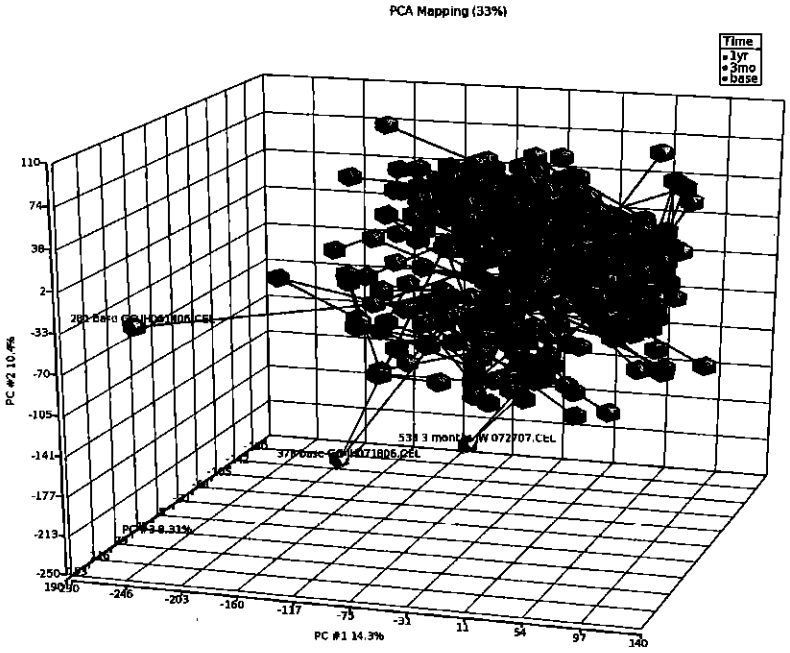
GO ID	Description	Annotated Genes in Total	Annotated Genes in List	Enrichment Score	FDR P-value
GO:0009617	response to bacterium	184	10	5.88	0.0072
GO:0006952	defense response	615	18	3.17	0.0072
GO:0006897	endocytosis	250	11	4.76	0.0072
GO:0010324	membrane invagination	250	11	4.76	0.0072
GO:0042742	defense response to bacterium	108	7	7.02	0.0178
GO:0010885	regulation of cholesterol storage	12	3	27.07	0.0325
GO:0016044	cellular membrane organization	375	12	3.46	0.0325
GO:0061024	membrane organization	376	12	3.46	0.0325
GO:0010878	cholesterol storage	13	3	24.99	0.0325
GO:0042632	cholesterol homeostasis	40	4	10.83	0.0634
GO:0055092	sterol homeostasis	40	4	10.83	0.0634
GO:0051707	response to other organism	308	10	3.52	0.0678
GO:0002764	immune response-regulating signaling pathway	44	4	9.84	0.0745
GO:0055091	phospholipid homeostasis	5	2	43.31	0.0745
GO:0030301	cholesterol transport	46	4	9.42	0.0745
GO:0015918	sterol transport	46	4	9.42	0.0745
GO:0042221	response to chemical stimulus	1163	22	2.05	0.0765
GO:0007015	actin filament organization	123	6	5.28	0.0765
GO:0006950	response to stress	1655	28	1.83	0.0765
GO:0055088	lipid homeostasis	50	4	8.66	0.0765
GO:0010887	negative regulation of cholesterol storage	6	2	36.09	0.0765
GO:0032715	negative regulation of interleukin-6 production	6	2	36.09	0.0765
GO:0042159	lipoprotein catabolic process	6	2	36.09	0.0765
GO:0010883	regulation of lipid storage	24	3	13.53	0.0800
GO:0002376	immune system process	1048	20	2.07	0.0803
GO:0010743	regulation of macrophage derived foam cell differentiation	25	3	12.99	0.0803
GO:0002768	immune response-regulating cell surface receptor signaling pathway	25	3	12.99	0.0803
GO:0006909	phagocytosis	57	4	7.60	0.0953

Data Supplement Table VIII. Self-reported Compliance in the Four Areas of CVD Risk Factor Modification

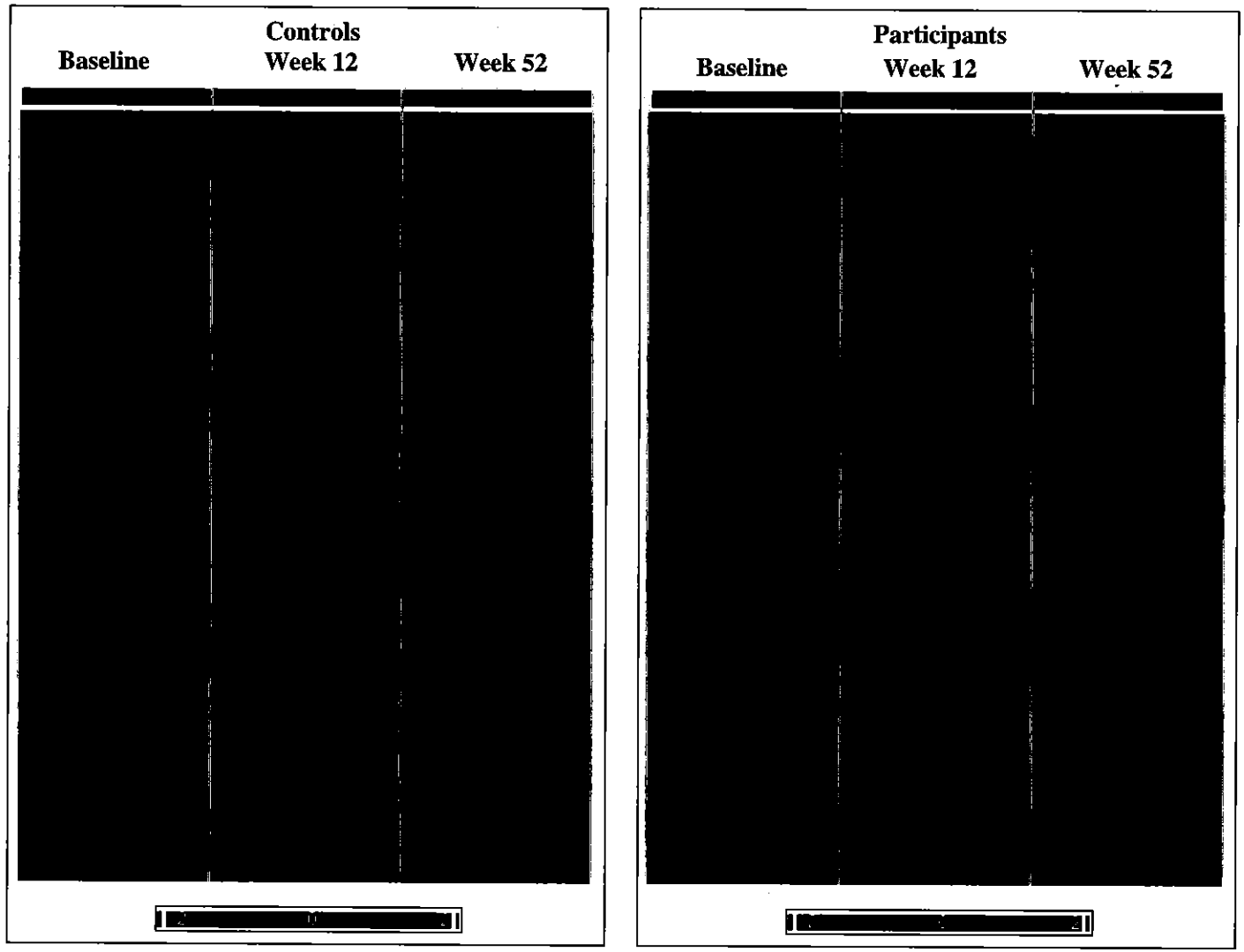
Area	Target Compliance Score	Average Compliance Score at 12 Weeks (SD)	Range	Percent of Participants Meeting Target at 12 Weeks	Average Compliance Score at 52 Weeks (SD)	Range	Percent of Participants Meeting Target at 52 Weeks
Diet	≥95	96.0 (4.4)	79-100	77.8	94.9 (9.1)	50-100	78.9
Exercise	≥100	121.9 (41.1)	31-250	77.8	98.3 (52.9)	0-350	54.0
Stress Mgt	≥100	99.6 (14.8)	41-171	66.7	94.4 (20.1)	14-165	46.0
Group	≥80	88.8 (9.6)	53-100	81.0	90.8 (11.9)	50-100	82.5



Data Supplement Figure 1. Histogram of RMA normalized log2 intensity



Data Supplement Figure 2. PCA plot colored by time point



Data Supplement Figure 3. Heat map depicting changes in global gene expression during intensive CVD risk reduction compared to matched controls.

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Improving Assessment of Cardiovascular Disease Risk by Using Family History

An Integrative Literature Review

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Background: Cardiovascular disease (CVD) is the number one killer in the United States. Although the causes of CVD are multifactorial, including genetic and environmental influences, it is largely a preventable disease. The cornerstone of CVD prevention is accuracy in risk prediction to identify patients who will benefit from interventions aimed at reducing risk. Nurse practitioners commonly perform CVD risk assessments and are well positioned to impact preventive therapy. Cardiovascular disease risk scoring systems currently in use substantially underestimate risk in large part because these do not include family history of premature CVD as a high-risk factor. **Purpose:** We sought to examine the state of evidence for the use of family history as a predictor in CVD risk stratification. **Conclusions:** A comprehensive literature search using the Medical Subject Headings terms of *family history of CVD, family history of premature CVD, risk assessment,* and *risk estimation* displayed 416 articles; a review of the titles and subsequent evaluation of the articles eliminated 392 references, leaving 24 for review. By incorporating family history in risk assessment, categorization of CVD risk improves substantially. The evidence demonstrates that family history is an independent contributor to risk appraisal and unequivocally supports its incorporation to improve accuracy in global CVD risk estimation. **Clinical Implications:** Underestimation of CVD risk leaves patients and providers misinformed, promoting the ongoing epidemic of chronic disease. Translating this evidence into practice by establishing a clinical algorithm that incorporates family history into risk prediction will standardize CVD risk assessment, improve the identification of high-risk patients, and provide the indicated aggressive care to prevent CVD.

KEY WORDS: cardiovascular disease, family history, nurse practitioners, primary prevention, risk assessment

Nurse practitioners caring for patients with cardiovascular (CV) disease (CVD) commonly perform assessments of long-term CVD risk. Such risk assessments are a cornerstone of preventive care because many risk factors respond to interventions and lower the patients' long-term risk.[1,2] Nurse practitioners are well positioned to have a major impact on patients' CVD risk through accurate, comprehensive assessments and corresponding interventions.

Although all care providers are taught to gather family history information, there is no systematic approach to applying such information to the patient's risk stratification.[3] Possible underestimation of CVD risk misguides patients and providers, leaving them unaware of potentially harmful health risks, many of which may be modifiable.

Cardiovascular disease risk assessment warrants a high degree of accuracy because current practice guidelines assign more aggressive goals for risk factor modification to patients identified as high risk compared with patients with low or moderate risk.[4] Although numerous CVD risk scoring systems have been developed and are widely used, many scores do not identify family history of premature CVD risk as a high-risk factor.[5]

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Family history is underused in risk assessment of CVD because of 2 main factors: (1) many traditional risk scoring systems do not incorporate family history (the National Cholesterol Education Program–Adult Treatment Panel III,[4] the Framingham Risk Score [FRS],[5] the European Societies of Cardiology,[6] and the Systematic Coronary Risk Evaluation[7]) and (2) clinical guidelines are vague and do not provide adequate guidance for the clinician to stratify risk by family history and thereby recommend appropriately aggressive interventions for the patient.[3,8] The purpose of this integrative literature review was to examine the evidence for inclusion of family history in CVD risk assessment to improve patient risk estimation.

Literature Search Methods

A comprehensive literature search was executed in the PubMed, Cumulative Index to Nursing and Allied Health, and EMBASE databases using the Medical Subject Headings terms of *family history of CVD*, *family history of premature CVD*, *risk assessment*, and *risk estimation*. The literature search was limited to studies reported during the time frame of years 1992 through 2012. The articles selected were peer reviewed, were published in English, and included adult participants. Manual searches were also performed by reviewing the reference lists of all selected articles for additional pertinent references.

The initial search using the terms *family history of CVD* or *family history of premature CVD* displayed 416 articles. When the terms *risk assessment* and *risk estimation* were applied, the number was reduced to 85. Limiting the search to adults further reduced the number of articles to 65. All citations were reviewed independently by 2 investigators to determine whether the references were appropriate for inclusion in the review of evidence. Any disagreements were resolved by discussion with a third investigator, and differences were resolved by consensus. Of the 65 publications, 37 were retrieved in full, having eliminated all others because these were not pertinent to the topic being investigated. Thirteen articles were opinion articles or editorials, and 24 articles were identified as appropriate and contributory for inclusion in the review.

Literature Search Results

The identified articles that were appropriate to include in the evidence review are listed in the Table. There were 9 observational studies, 10 cross-sectional studies, 4 case-control cross-sectional studies, and 1 randomized controlled trial (RCT).

Literature Review Findings

The research articles in the Table are listed in chronological order of publication and categorized by study design. The populations studied, the sample sizes, and the limitations of the studies are noted.

Three of the earliest studies used observational designs that followed large patient populations for up to 26 years.[9–11] These studies found that a positive family history of CVD is a powerful predictor of incident CVD, independent of other risk factors. In the Swedish Twin Registry, death from CVD in 1 twin before the age of 55 years conferred dramatic increases in risk for death from CVD in the surviving twin (8-fold and 15-fold increased risk for men and women, respectively).[9] In the other 2 studies, the degree of risk associated with CVD in a first-degree relative ranged from 41% increased[10] to as high as 250% increased.[11] Furthermore, maternal history of CVD was demonstrated to be as important as paternal history.[11] These studies did manifest limitations in their design. Specifically, the Swedish Twin Registry was a retrospective observational study relying on death certificates with diagnoses coded by the International Classification of Diseases. The Atherosclerosis Risk in Communities study[10] demonstrated selection bias because the nonresponder portion of the population had lower educational achievement and tended to have less healthy lifestyles. This selection bias might overestimate or underestimate the impact of family history, in that people with higher cardiac risk estimates may not have been included in the study. The Physician Health Study and the Women's Health Study were limited, in that family history information was collected on parental CVD but not on sibling history.[11]

An early cross-sectional study, the Stockholm Heart Epidemiology Program, reported the synergistic effects of family history as a predictor of CVD when used in women in combination with current smoking history and a low-density lipoprotein/high-density lipoprotein quotient[12] of higher than 4.0. In men, family history showed predictive synergy with diabetes mellitus.

A more recent analysis from a large-scale case-control study, the INTERHEART study,[13] established parental family history of myocardial infarction (MI) as a robust predictor that was significantly associated with risk for MI, independent of 9 major CVD risk factors (abnormal lipids, smoking, high blood pressure, diabetes, abdominal obesity, psychosocial factors, physical activity, fruit and vegetable consumption, and alcohol consumption). This relationship was consistent across all world regions, income, age groups, and gender analyzed in the study, including all FRS risk groups.[13]

Five cross-sectional studies using coronary artery calcium (CAC) scores measured by electron beam computed tomography (EBCT) were published between 2004 and 2007 by 3 different investigator groups in 5 study populations.[14–18] Each of these studies demonstrated a strong association of high CAC scores with family history. A positive sibling history was found to be more strongly associated with a high CAC score

TABLE Results and Summary of Integrative Literature Review

Author and Date	Evidence Type	Sample and Sample Size	Study Findings	Conditions and Limitations
Marenberg et al[9] 1994	Retrospective observational	21,004 Swedish twins, 56% women, followed for 26 y	When 1 twin died of CHD before 55 y of age, hazard of death from CHD was 8.1 in men and 15.0 in women among homozygotes and 7.1 in men and 2.6 in women among dizygotes, independent of other risk factors.	Data from death certificates. Not all conventional risk factor data were available. Effect of lifestyle factors cannot be ruled out. Homogeneous population.
Li et al[10] 2000	Observational	ARIC Study (10,589 whites, 3,398 African Americans), aged 45–64 y, from US communities	Family risk score predicts incident CHD independent of selected risk factors (41%–68% higher risk with FH).	Selection bias because nonresponders were less educated and had less healthy lifestyles. Family history by self-report.
Friedlander et al[24] 2001	Cross-sectional case-control	107 women with first acute MI, aged 18–44 y, in Western Washington State and 526 women similarin age with no history of CVD	Relative risk of MI in first-degree relatives is 1.96. After controlling for established risk factors, sibling MI history showed an OR of 5.17.	Small sample size. Family history by self-report.
Sesso et al[11] 2001	Observational	Physician Health Study (22,071 men); Women's Health Study (39,867 women)	Premature paternal history is an important, independent predictor of CVD in both men and women. Maternal history of MI seems to predict CVD at least as strongly as paternal history of CVD and also at older ages of maternal age. FH in both parents yields RR of 1.98 for MI in men and RR of 2.49 in women.	Both MI and stroke were studied. Only parental history was collected. Family history by self-report.
Leander et al[12] 2001	Cross-sectional case-control	SHEEP Study (1,091 men and 531 women), aged 45–70 y, residents of Stockholm surviving 28 d from first acute MI	Family history of CHD is a strong risk factor of MI in both genders and is synergistic with other CV risk factors.	Recall bias. Study design excludes fatal cases of MI. Family history by self-report.
Andresdottir et al[27] 2002	Observational cohort	19,390 randomly selected residents of Reykjavik, Iceland, mean follow-up of men, 18 y; women, 19 y	After factoring in influence of traditional risk factors, HR of CHD was 1.66 for men and 1.64 for women with a first-degree relative with an MI, compared with participants without a family history.	Homogeneous population. Family history by self-report.
Wang et al[20] 2003	Cross-sectional	1,662 patients in the Framingham Offspring Study (mean age, 57 y; 51% women)	After adjustment for CV risk factors, CIMT was greater for participants with at least 1 parent with premature CHD compared with those without parental history.	Outcome measure was CIMT, not angiography. Homogeneous population—mostly white.
Lloyd-Jones et al[21] 2004	Observational	2,302 participants in the Framingham Offspring Study (mean age, 44 y; 51% women) followed for 8 y	Among participants who had 1 parent with premature CVD, OR of CVD event was 2.6 in men and 1.7 in women.	Did not control for medication use. Homogeneous population—mostly white.
Nasir et al[14] 2004	Cross-sectional	MESA Study (8,549 participants getting EBCT scan, 69% men); mean (SD) age, 52 (9) y	Family history of CHD is highly associated with coronary artery calcification. Sibling history of CHD is more strongly associated (OR, 2.5) with subclinical atherosclerosis than parental history.	Family history by self-report.
Taylor et al[15] 2004	Cross-sectional	PACC Study, Walter Reed Army Medical Center (1,619 asymptomatic healthy men); aged 40–50 y	The prevalence of any coronary artery calcification was 19.3% in participants with no family history (n = 1102), 26.6% in those with a first-degree family history (n = 203; 12.5%), 26.5% in those with a second-degree family history (n = 215, 13.3%), and 30.3% in those with both (n = 99, 6.1%, $P = 0.003$). Odds ratios for coronary artery calcification associated with a first-degree (1.49; $P = 0.026$) and a second-degree (1.41; $P = 0.049$) family history of CHD were similar.	Homogeneous population (healthy men from military). Family history by self-report.

(continues)

TABLE Results and Summary of Integrative Literature Review, continued

Author and Date	Evidence Type	Sample and Sample Size	Study Findings	Conditions and Limitations
Michos et al[19] 2005	Cross-sectional	Johns Hopkins Sibling Study (102 consecutive asymptomatic female siblings, sisters of proband hospitalized with documented premature CHD), mean (SD) age, 51 (7) y	Traditional risk factors may fail to identify a large proportion of women with subclinical disease. These women would not meet criteria for primary prevention by AHA guidelines. Significant subclinical atherosclerosis is common in women with positive family history. The FRS underestimates the true burden of CAD in women.	Small sample size. Outcome measure was CAC, not angiography.
Murabito et al[22] 2005	Observational	Framingham Offspring Study, (2,475 members without CVD) 30 y or older, with at least 1 sibling in the study	Sibling CVD confers increased risk for CVD events (OR, 1.55) independent of premature parental CVD and established risk factors.	No risk factor data on siblings who declined enrollment. Mostly white cohort and therefore not generalizable.
Michos et al[16] 2005	Cross-sectional	EBCT scanning facility, Columbus, Ohio (6,141 asymptomatic patients without known CHD at the time of screening)	Family history of premature CHD plus 2 risk factors predict higher burden of CAD (OR, 1.77). Despite being in a low-risk group, 32% of women had significant atherosclerosis (>75th percentile).	Family history of CHD and risk factors were self-reported. Blood sugar was not recorded.
Michos et al[17] 2006	Cross-sectional	EBCT scanning facility, Columbus, Ohio (2,447 consecutive asymptomatic women without diabetes)	The FRS classified participants as low risk even when CAC was identified.	Outcome measure was coronary artery calcification, not angiography. Family history by self-report.
Scheuner et al[25] 2006	Cross-sectional	HealthStyles 2003 annual mail survey, (4,035 respondents) 60% women, 72% white, mean age of 48.8 y	Strong family history of CHD is associated with 5-fold increased risk for early-onset CHD; moderate family history of CHD, 2-fold increased risk.	Self-reported survey lacks objective validation of data. Confounding by survival. Family history by self-report.
Nasir et al[18] 2007	Cross-sectional	MESA Study (6,814 women and men), aged 45–84 y, recruited from 6 US communities	In asymptomatic men/women with low and intermediate risk, family history (parental and sibling) is associated with higher prevalence of coronary artery calcification, independent of other risk factors and the FRS. Family history of premature CHD confers risk for coronary artery calcification higher than the 75th percentile, OR of 1.93.	Second examination not blinded to family history or first examination. Family history by self-report.
Cipriani et al[23] 2010	Cross-sectional case control	Multicenter study in Italy of 2,016 patients with 11,696 relatives and 1,757 controls with 8897 relatives	Among patients surviving their first MI before the age of 46 y, risk for early-onset MI among siblings (HR, 1.7) was higher than among parents (HR, 0.9).	No traditional risk factors measured. Family history by self-report.
Rubinshtein et al[31] 2010	Cross-sectional	Mayo Clinic Study (1,063 consecutive patients referred for angiography), mean (SD) age of 50 (12) y.	Impaired coronary microcirculation function may be present, with low to intermediate FRS without obstructive CHD. Other traditional risk factors have no association (eg, CRP).	Referral bias. Of the patients, 64% were women without significant CHD. Family history by self-report.
Sivapalaratnam et al[28] 2010	Observational	European Prospective Investigation of Cancer—Norfolk region, United Kingdom (22,841 participants, 55% women, aged 40–79 y)	Positive family history was an independent risk factor of CHD (HR, 1.74).	Coronary heart disease identified by death certificates and hospital reports. Family history by self-report.
Chow et al[13] 2011	Cross-sectional case-control	INTERHEART multinational study (12,149 patients/14,467 controls)	Parental history of MI confers risk independent for 9 traditional risk factors, consistent across world regions, income, age, and gender. Odds ratios for risk for MI were 1.88 in men and 1.62 women.	Recall bias noted. Only parental history was collected. Family history by self-report.
Walizer et al[26] 2011	Cross-sectional	BATTLE Study, Walter Reed Army Medical Center (93 asymptomatic patients at high risk by CIMT)	The FRS classified 75% of participants as low risk even when CIMT showed subclinical atherosclerosis. A total of 60% of patients had a positive family history of CVD.	Outcome measure was CIMT, not angiography. Family history by self-report.

(continues)

TABLE Results and Summary of Integrative Literature Review, continued

Author and Date	Evidence Type	Sample and Sample Size	Study Findings	Conditions and Limitations
Qureshi et al[32] 2012	Randomized, matched cluster	748 participants with no previous CAD, aged 30–65 y	Number of persons classified as having high CV risk increased by 41% in the intervention group (from 49% to 69%) compared with a 6% increase in the control group (36%–38%).	Homogeneous population. Family history by self-report.
Bachmann et al[29] 2012	Observational	A total of 49,255 men from the Cooper Center longitudinal study, Cooper Clinic, Texas: preventive medical practice focusing on periodic health examinations. Patients were referred to the study by employer, physician, or self.	After adjustment for traditional risk factors, premature family history was associated with CHD mortality of 10–20 y (HR, 1.59) and more than 20 yrs (HR, 1.43). Compared with men without family history, premature family history was associated with a 50% higher lifetime risk for CVD mortality (21% vs 14%).	Broad definition of family history (including grandparents, aunts, uncles). Family history by self-report.
Ranthe et al[30] 2012	Observational	All residents of Denmark from 1950 to 1978 in database; almost 4 million persons followed for 89 million person-years	Premature CVD in any first-degree relative increases risk for developing early CVD by 72% compared with the risk in persons with no history of premature CV death among first-degree relatives.	Data collected from database, potential for misclassification of CVD status.

Abbreviations: AHA, American Heart Association; ARIC, Atherosclerosis Risk in Communities; BATTLE, Better Adherence to Therapeutic Lifestyle Change Efforts; CAD, coronary artery disease; CRP, c-reactive protein; FH, family history; OR, odds ratio; HR, hazard ratio; PACC, prospective army coronary calcium; SHEEP, Stockholm Heart Epidemiology Program.

than parental history alone, and family history in both sibling and parent yielded the strongest association of all.[14] Family history in a first-degree relative was more strongly associated with CAC than family history in a second-degree relative, but a positive family history in both first- and second-degree relatives had the strongest degree of association.[15] In a population referred by a physician for EBCT, a positive family history of coronary disease acted synergistically with other risk factors to magnify the association with a high percentile CAC score.[16] This finding was demonstrated in a population of asymptomatic women who were generally at low risk for CVD. In asymptomatic women without diabetes, the performance of the Framingham risk estimation (FRE) was evaluated by CAC score. The majority of (84%) women with significant CAC (>75th percentile) were classified as low risk, and approximately half of the low-risk women with a family history of premature coronary disease had significant CAC.[17]

The final cross-sectional study using CAC as its main measure is the Multi-Ethnic Study of Atherosclerosis (MESA) trial.[18] This study sought to examine whether asymptomatic individuals with positive family history of premature CVD have an increased atherosclerotic burden in the low- and intermediate-risk categories based on the traditional FRS. Family history was significantly associated with coronary artery calcification in all ethnic groups. The prevalence of coronary artery calcification was higher in those with family history compared with those with no family history in the low-risk (35% vs 23%, $P < 0.0001$) and in the intermediate-risk (70% vs 60%, $P = 0.01$) categories.[18] The authors state that family history in middle-aged adults should be considered for quantification of risk and incorporated with existing risk prediction algorithms for aggressive primary prevention such as intensified weight loss and pharmacotherapy (lipid-lowering, aspirin, and antihypertensive therapy).[18] All 5 of the cross-sectional coronary artery calcification studies had the limitation of determining family history by self-report rather than from the objective examination of medical records.

The Johns Hopkins Sibling Study had a unique study design, in that it enrolled 102 asymptomatic women who were sisters of patients hospitalized for documented premature coronary disease.[19] The 102 enrolled participants underwent assessment using the FRE. Low-risk FRE was assigned to 98 (98%) of the 102 participants, and moderate risk was assigned to the remaining 2 participants (2%). However, 40% of these participants had detectable coronary artery calcification. Significant subclinical atherosclerosis was found in 32% of the participants, and 17% had CAC scores that ranked higher than the 90th percentile.[19]

The Framingham Offspring Study has developed a cohort of participants whose data have been analyzed using several different study designs. Three publications

have correlated newly gathered data from these participants, who are offspring of the original Framingham participants. In a cross-sectional study, carotid intima media thickness (CIMT) of 1662 offspring participants was analyzed by ultrasound. Participants who had 1 parent with premature coronary heart disease (CHD) were found to have greater CIMT values compared with participants with no parental history.[20] In another look at the offspring, this time an observational design following 2302 participants for 8 years, participants who had 1 parent with premature CHD were 1.7 (women) to 2.6 (men) times more likely to experience a CVD event.[21] A third offspring study examined a cohort of participants who were selected on the basis of the fact that at least 1 sibling was enrolled and followed in the Framingham Heart Study.[22] Baseline risk factors and incident events for 8 years were much higher in the sibling with CVD group than in the sibling without CVD group (odds ratio, 1.55). The study also demonstrated that sibling CVD conferred increased risk for future CVD events above and beyond the established risk factors and parental CVD.

A cross-sectional case-control design was used in a multicenter Italian study that enrolled 2,016 patients with 11,696 relatives and 1,757 controls with 8,897 relatives.[23] The analysis showed that, among the patients surviving their first MI before the age of 46 years, the risk for early-onset MI was higher among siblings (hazard ratio [HR], 1.7) than among parents (HR, 0.9).

In a population from Western Washington State, 107 women aged 18 to 44 years with their first acute MI were compared with 526 women similar in age who served as control participants.[24] A detailed questionnaire elicited history of MI in first-degree relatives. The rate of MI was twice as high in first-degree relatives of the patients compared with the controls.

The Health Styles annual mail survey reports that family history may be especially useful in younger populations whose traditional risk factors seem benign.[25] There were 4035 respondents, 60% women, with a mean age of 48.8 years. The authors report a 2- to 5-fold increased risk for early-onset CVD based on degree of severity in family history. The authors propose that, to address the barriers in existing guidelines, national guidelines should view family history as a tool that will (1) aid in the identification of people with significantly increased disease risk, attributable, in part, to genetic factors; (2) improve early detection and prevention efforts for people with increased familial risk; and (3) facilitate treatment algorithms, with messages tailored to the level of familial risk.

The inadequate performance of the FRS was further demonstrated by the Better Adherence to Therapeutic Lifestyle Change Efforts trial.[26] In this analysis of 93 asymptomatic patients at a military hospital in Washington, DC (59% women; mean age, 54 years), all patients were identified as high risk for heart disease by CIMT measurement, a surrogate measure of atherosclerosis. Of these high-risk patients, 75% were categorized as low risk by the FRS. Of note, 60% of the 93 patients had a positive family history of CVD.

Four observational studies are noteworthy for their inclusion of enormous study populations. These studies range in size from nearly 20,000 randomly selected Icelanders followed for up to 19 years,[27] to nearly 23,000 UK citizens in the European Prospective Investigation of Cancer study,[28] to nearly 50,000 participants in the Cooper Center longitudinal study,[29] to almost 4 million residents of Denmark followed for 89 million person-years.[30] These studies all demonstrated similar findings of a substantial risk for the development of CHD[27,28,30] (HR, 1.6–1.7) or CHD mortality[29] (HR, 1.4–1.6), conferred by a positive family history of CVD.

A novel measure was used in a study from the Mayo Clinic to assess the predictive value of family history in CVD.[31] In a large cohort of patients without obstructive coronary disease by coronary angiography, coronary vasoreactivity was evaluated with intracoronary bolus injections of adenosine followed by infusions of acetylcholine, with consequent measures of coronary diameters and coronary blood flow. The concept of the study relied on the notion that there can be functional abnormalities at the level of coronary microcirculation at early stages of coronary atherosclerosis in the absence of obstructive disease. The outcome measure served as a proxy for CVD events or CV death. The study reported that, in a multivariable analysis, positive family history of coronary disease was a significant independent predictor ($P = 0.04$) of reduced coronary flow reserve, a precursor to the development of atherosclerosis.

The feasibility of systematically collecting family history information for CVD risk was demonstrated in the primary care setting.[32] In a matched-pair cluster RCT, control participants had the usual FRS-based CVD risk assessment with family history, as usually recorded in their medical records. Intervention participants had the usual CV risk assessment but also completed a family history questionnaire. There was a near universal completion rate (98%), and the number of participants found to be at high risk for CVD increased by 41% by using the family history questionnaire. Incorporating systematically collected family history information was demonstrated to be feasible and cost-effective.

Discussion

This review of the evidence clearly reveals that family history is an important independent predictor of CVD, as demonstrated in 19 separate studies.[1–13,20,22,24,27–29] Of course, it is possible that this impression results from publication bias because positive associations tend

to make it to publication, whereas negative findings are often left unpublished. However, a number of observations argue against publication bias and validate the important role of family history in risk estimation.

First, every study that addressed the utility of family history in CVD risk assessment uniformly agrees with the central finding that family history adds to commonly used risk prediction models. Frequently used descriptors from these studies are that family history is an independent predictor and that family history is synergistic with other risk factors in improving accuracy of risk assessment.

Second, a variety of different outcome measurements and different study designs supported the utility of family history. The outcome measures and study designs include self-reported events by mail survey,[25] anatomical assessments with CAC scores[14–18] and CIMT[20,26] in cross-sectional surveys, case-control cross-sectional studies in patients with events,[12,13,23,24] cross-sectional evaluations of siblings of patients with events,[19] prospective observational studies of incident events,[10,11,21,27,29] assessment of vasoreactivity during angiography,[31] and an RCT.[32] Despite the variety of outcome measures and study designs, every study validated the utility of family history as an independently predictive risk factor.

An area of concern with published articles on the effect of family history on CVD risk is the use of heterogeneous definitions of what constitutes a positive family history. The spectrum of definitions ranges from any CV event in a related person including second- and third-degree relatives at any age to specific cardiac events in first-degree relatives younger than 50 years. Because of the variability in definitions, practitioners are unlikely to understand the predictive value and relevance of family history findings. The lack of a precise definition is a global problem in the assessment of family history.

Naturally, the degree of predictive power wielded by family history varied greatly depending on the characteristics of the population included in the study. The INTERHEART study showed predictive value of family history, independent of other risk factors consistent across world regions, income, age, and gender.[13] However, other studies emphasize differences in the contribution of family history by gender and race.[10,11] Specific studies suggest that family history is of particular value in the assessment of CVD risk for both women[17,19,24] and the young.[15,21,24,25] These 2 groups have traditionally been scored as low risk for CVD by scoring systems using conventional risk factors. Identifying these groups as targets for early intervention is critical because the process of CVD develops for decades and intervention is most effective when applied as early as possible in the cascade of events. Identifying risk for developing CVD in these subgroups of the population could confer great benefit to the preventive efforts.

Limitations

This literature review was limited to publications in English and may have therefore missed substantial work in other languages.

There are a number of limitations in the studies reviewed and discussed. Many of the studies are subject to referral bias; that is, participants included for analysis in the studies are not the same as participants who are not included. Typically, refusal to participate in research is behavior associated with persons whose educational level is low, whose general health behavior is unfavorable, or who are too ill to participate. This implies that participants who might benefit the most from efforts at risk assessment and risk reduction are not being systematically included in studies performed to date.

Most of the publications included in this review of evidence used family history according to participant recall. No study used formal medical record reviews of family history except for sibling and offspring studies in which participants were identified by sibling or parents with a CVD event.[19,22] Errors in recall are understandable and expected but do limit the reproducibility of results. Nevertheless, participant recall most closely mimics the method used to obtain family history in clinical settings and may therefore be the most appropriate method to use for clinically useful studies.

Because a broad variety of outcome measures and study designs were used to assess CV risk, such as events, coronary artery calcification, CIMT, and vasoreactivity, it is difficult to compare findings between studies. The heterogeneity of measures and study designs may be partly responsible for the wide range of statistical estimates of the impact of family history on an individual's risk; that is, the impact size of a positive family history varies enormously between reports. The heterogeneity also makes it impossible to merge data from multiple studies in a formal meta-analysis to determine the global quantitative impact of a positive family history on the degree of increased risk. However, the heterogeneity of study designs may also be a potential strength of this review, in that multiple different study designs all seem to point to the same conclusion that family history is an invaluable, potent, and independent predictor of CVD risk. Irrespective of the road taken, the journey ends with the same affirming conclusion, strengthening the validity of the findings.

Another limitation in the studies reviewed is that most of the studies were not undertaken with CVD risk and family history as the purpose of the study a priori. Most of the studies reported on data collected under the auspices of another protocol for another purpose. Some of the studies were population subsets of an original protocol. The implication is that these data may produce interesting and valuable findings, but more definitive studies should be pursued with CVD risk and

family history as the prime objective to collect a truly trustworthy data set to answer the question.

Implications for Practice

Despite consistent evidence supporting family history as a known risk factor of CVD, few standards address how this information should be collected, interpreted, and applied to clinical practice. Family history is not commonly used to counsel patients about risks or preventive behaviors largely because of ambiguity in the guidelines.[3,33] These findings underscore the importance for the use of family history as an accessible and inexpensive standardized tool for risk-based interventions. For example, although the third Adult Treatment Panel III of the National Cholesterol Education Program guidelines[4] (2004) recommends the assessment of family history, there are currently no guidelines for clinical decision making if the patient has positive family history in the absence of other CVD risk factors.[1] Because of this confusion, a great deal of variability exists in clinical practice. When CVD risk is underestimated, the clinical impact is significant because miscategorization of risk misleads providers to set less aggressive treatment goals, which, in turn, causes patients to follow recommendations that may be suboptimal for their true risk level.

A cross-sectional analysis reveals that clinicians need direction in using family history to improve health by risk assessment algorithms and guidelines.[34] This recent study of Kaiser Permanente clinicians in Oregon reported that family history information would be more useful if it were integrated into algorithms and tools already used in clinical decision making, such as the FRS. In addition, the authors recommend that family history information be used to identify persons who are at an increased risk for CVD and who may be receptive to and benefit more from CVD preventive interventions than patients with no family history. The presence of family history may motivate patients who do not have CVD but are at high risk for CVD to engage in healthy, potentially protective behaviors.[32]

In a discussion of strategies for CVD prevention, cost-effectiveness of family history data collection as accessible and costing between $1 and $3 through Internet collection is compared with the estimated $200 for laboratory data used to measure other risk factors.[35] The article recommends combining family history information with clinical assessment for more intensive intervention. Screening for family history also allows patients with borderline risk factors to be aware of their CVD risk status. Even high-risk patients should be reassured that specific lifestyle changes and preventive therapies help to reduce risk.

Risk assessment in primary care is critical because it determines and guides the intensity of therapeutic intervention. First, causal risk factors should be explored, similar to those collected for the FRS.[27] Second, predisposing risk factors such as family history of CVD should be assessed. In addition, advance practice nurses who receive education in behavioral change strategies, lifestyle interventions, and management of risk factors can implement the aggressive therapy necessary for these patients such as adherence to cholesterol treatment guidelines.[36] Prevention programs promoting family history evaluation have been shown to be more successful than those that do not. Furthermore, advance practice nurses can address erroneous beliefs related to family history, such as fatalism; motivate when family history is present; and adjust the severity for treatment recommendations.[32]

Summary and Recommendations for Future Research

Despite the strong evidence, gaps in knowledge remain. Without large RCTs that longitudinally measure hard outcomes, such as CVD events, it will be difficult to evaluate true risk prediction. Such studies are necessary to distinguish between the diagnostic or predictive value of family history and CVD. Evaluation of family history in a large sample size over time can also provide knowledge regarding gender differences and specific cut points when the risk is increased, such as with postmenopausal women.

Additional studies are also necessary to test the utility of family history when combined with existing, well-validated risk scores such as the FRS. There are numerous family history risk prediction models that have been proposed; however, the studies lack power, making it difficult to identify applicability to the population at large. In addition, many scores have added other emerging risk factors to these predictive equations, making it difficult to assess family history separately.

There are also gaps in knowledge regarding the barriers to using family history in CVD risk assessment. For example, is the root of the problem the perception of family history, knowledge of how to incorporate the information, or inadequate guidelines to follow for preventive therapy? It is unclear why there is a lag between guideline development and adoption of guidelines into clinical practice.

Review of literature for strength and quality reveals that family history is a missing component of CVD risk appraisal, and its inclusion could improve accuracy in global CVD risk estimation. Recommendations for a process change are made on the basis of the evidence that family history data are accessible, inexpensive, and personalized. The research evidence strongly supports using a clinical algorithm that recognizes family history of premature CVD as an independent high-risk factor, in addition to the FRS or incorporated within

Clinical Pearls

- Literature review unequivocally supports the inclusion of family history as a strong and independent risk factor of cardiovascular risk estimation.
- Although clinical guidelines recognize family history as an indicator of heightened risk, these guidelines do not provide nurse practitioners with clear direction for clinical decision making.
- A clinical algorithm for inclusion of family history would standardize preventive health practices, improve the identification of high-risk patients, and provide preventive interventions that address patients' accurate risk.

the FRS. Translation of this research into practice would help providers such as nurse practitioners to assess family history of CVD with a systematic process and to recommend appropriate aggressive preventive care for patients in this high-risk category.

The opportunity to capture patients at risk for CVD, who are otherwise overlooked by traditional methods, and to provide them the awareness to protect themselves before overt disease presents is supported by robust evidence. This evidence must not be overlooked. Furthermore, the cost of preventive care for patients detected earlier in the progression of disease would be far less than that after an acute CVD event has taken place.[1]

Ultimately, the implementation of a clinical algorithm for family history of premature CVD would translate the evidence to standardize preventive health practices, improve the identification of high-risk patients, and provide this susceptible population with the needed primary preventive care to combat the number 1 killer of both men and women in the United States.

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Cardiometabolic risk reduction in an intensive cardiovascular health program

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Abstract *Background and aims:* Insulin and leptin are important markers of insulin resistance and vascular inflammation in metabolic and cardiovascular diseases. This study evaluated changes in circulating levels of insulin and leptin during a cardiovascular health program to improve our understanding of cardiometabolic risk reduction.
Methods and results: Participants ($n = 76$) completed a prospective, nonrandomized program designed to stabilize or reverse progression of coronary artery disease through dietary changes, exercise, stress management, and group support. Controls ($n = 76$) were matched to participants based on age, gender, and disease status. Traditional cardiovascular risk factors were assessed at baseline, 12 weeks, and 52 weeks by standard methods. Dietary data were collected by 72-h recall and evaluated by Food Processor® v8.4.0. Ultrasensitive insulin and leptin levels were measured by radioimmunoassay. Participants successfully reduced their total caloric intake from >2000 calories per day to ~1700 calories per day ($p < 0.05$ compared to controls), lowered daily fat intake by >60% ($p < 0.001$ compared to controls), and increased carbohydrate intake by >30% ($p < 0.001$). Repeated-measures ANOVA indicated significant beneficial changes ($p < 0.001$ compared to controls) in plasma insulin (−19%) and leptin (−33%) during the lifestyle program, as well as improvement in traditional cardiovascular risk factors. Response was similar between men and women for most risk factors and was not markedly influenced by medication use.
Conclusion: Lifestyle changes focusing on diet, physical activity, and stress reduction can successfully modify both cardiovascular and metabolic risk factors, with the potential to mediate cardiometabolic risk through beneficial anti-inflammatory and anti-oxidative effects on the vasculature.

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Introduction

Insulin and leptin represent two important and well-characterized markers of insulin resistance and vascular inflammation in metabolic and cardiovascular diseases (CVD). Insulin is a polypeptide hormone that affects the vascular endothelium by modulating glucose homeostasis and glycogen synthesis [1]. Fasting insulin levels have increased dramatically in non-diabetic adults over the past two decades, often developing as a consequence of resistance to the action of insulin in peripheral tissues [2]. Hyperinsulinemia has been linked to dyslipidemia, impaired glucose regulation, and hypertension [3], as well as overall risk for cardiovascular mortality [4].

Leptin is an adipocytokine secreted by white adipose tissue that functions mainly in energy balance and metabolism, but plays an important role in vascular physiology through interactions with the vascular endothelium [5,6]. High circulating levels of leptin may accelerate atherosclerosis and contribute to CVD risk by inducing oxidative stress on endothelial cells [7] and impairing arterial reactivity [8]. Clinical studies have shown that high leptin contributes to CVD risk in the general population and is associated with myocardial infarction and coronary events, independent of traditional cardiovascular risk factors [9,10].

Insulin resistance, vascular inflammation, and oxidative stress play important roles in endothelial dysfunction. Pharmacologic therapies to improve endothelial function show marked variability in their ability to lower circulating markers of inflammation [11], and are often used in combination to be most effective in reducing inflammation and oxidative stress. An alternative approach for treating patients with high cardiovascular risk involves lifestyle modification to reduce traditional CVD risk factors and slow or reverse progression of coronary atherosclerosis [12]. Lifestyle programs focusing on nutrition and exercise can improve endothelial function and enhance insulin sensitivity, in part by reducing markers of systemic vascular inflammation and insulin resistance [13].

Insulin and leptin have important effects on vascular biology, but may function through different molecular pathways — insulin through metabolic pathways and leptin through inflammatory and thrombogenic factors [14]. We investigated the impact of an intensive cardiovascular health program on circulating levels of insulin and leptin to improve our understanding of cardiometabolic risk factor reduction by (1) measuring changes in physiological risk factors for CVD throughout a year-long cardiac health program and (2) assessing response of insulin and leptin and relating changes in these inflammatory markers to improvement in vascular health.

Methods

Study population

The intervention group consisted of 76 white men and women who completed a prospective, nonrandomized program to stabilize or reverse progression of coronary artery disease (CAD) through dietary changes, exercise, stress management, and group support. Eligibility criteria were (1) a diagnosis of CAD, including acute myocardial infarction, bypass surgery, stent placement, stable angina, angioplasty, or evidence of $\geq$50% luminal narrowing on coronary angiogram; or (2) two or more CAD risk factors such as high blood pressure (BP) defined as systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg, high total cholesterol (>200 mg/dL), physician diagnosed diabetes, obesity — body mass index (BMI) $\geq$30 kg/m^2, or family history of heart disease in parents or siblings. Physician approval, motivation to commit to following the guidelines of the program, and successful abstinence from smoking for at least three months prior to enrollment also were part of the acceptance criteria.

Controls ($n = 76$ white men and women) were matched to participants based on gender, age at baseline within five years, and CAD status (overt CAD or risk factors) using a prospective individual matching strategy to achieve a balanced distribution of risk factors between intervention participants and controls in nonrandomized clinical trials [15]. Controls receiving only standard care from their primary physicians underwent identical examinations at baseline, 12 weeks, and 52 weeks, but did not participate in the program or receive healthy lifestyle information.

This study was approved by the Institutional Review Board at Windber Medical Center. All participants voluntarily enrolled in the program and provided written informed consent.

Intervention

The lifestyle program included four components: (1) low-fat vegetarian diet (<10% of calories from fat); (2) 180 min/week of moderate aerobic exercise; (3) 1 h of stress management each day; and (4) two 1-h group support sessions per week for the first 12 weeks and one group session per week during the remainder of the year [16]. Adherence was self-reported by summarizing diet (fat, carbohydrate, protein intake), exercise (frequency and duration), stress management (frequency and duration), and group support (frequency of meeting attendance) for each day. Program staff reviewed compliance forms weekly and provided immediate feedback to participants on progress and guidance for improving adherence.

From January 2004 to February 2009, approximately 35 participants or controls were enrolled each year in separate cohorts of ~12 individuals per cohort. The dropout rate was ~32% ($n = 53$) among participants in the program, likely attributable to the magnitude of lifestyle changes required.

Physiological measures

Data collection and reporting followed recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group [17]. Clinical examinations conducted by physicians or trained personnel at baseline, 12 weeks, and 52 weeks collected information on age, gender, ethnicity, smoking status, cardiovascular history, and medication use. Height and weight measurements were used to calculate BMI. Blood pressure was recorded using a mercury sphygmomanometer on the arm of

seated, relaxed subjects. General endurance was determined by a graded treadmill exercise test that estimated the volume of oxygen each participant could consume (VO_2 max; ml/kg/min) based on exercise intensity, duration, and body weight (Bruce score) [18]. Assays for standard high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, and triglycerides were conducted using the AEROSET™ clinical chemistry system (Abbott Laboratories, Abbott Park, IL).

Insulin and leptin measurements

Fasting blood samples for standard insulin and leptin analysis were obtained at each examination and placed directly on ice. Within 1 h of collection, plasma aliquots were isolated by centrifugation and stored at −80 °C. Ultrasensitive insulin (μU/ml) and leptin (ng/ml) levels were measured in duplicate on freshly thawed plasma samples by radioimmunoassay (Millipore, Billerica, MA) at the Johns Hopkins Bayview Clinical Research Unit. Inter-assay coefficients of variation (CV%) were 3.27 for insulin and 3.81 for leptin.

Dietary composition

Participants and controls completed a self-reported 72-h dietary recall questionnaire at each examination, recording their total dietary intake for breakfast, lunch, dinner, and snacks over three consecutive days. Participants reported specific food items and drinks consumed, portion sizes, preparation methods, and location (home or away). Food Processor® v8.4.0 (ESHA Research, Salem, OR) was used to determine daily caloric intake and nutrient composition.

Statistical analysis

Statistical analyses were conducted using SPSS version 15.0 and JMP® version 9.0; p values <0.05 were considered significant. Prior to analysis, normality of the outcome data was determined by Lilliefors test, and natural log-transformations were used for variables with non-normal distributions. Potential differences in baseline measures among participant cohorts and among control cohorts were examined by analysis of variance (ANOVA). As no significant cohort-to-cohort variability at baseline was detected, all intervention and all control cohorts were, respectively, combined in subsequent analyses.

An independent samples t-test, or nonparametric Mann–Whitney U test if data remained non-normally distributed after natural log transformation, was used to compare baseline characteristics between intervention participants and controls. Repeated-measures ANOVA was used to compare changes in CVD risk factors at 12 weeks and 52 weeks between intervention and control groups. Independent samples t-tests (two-tailed) then identified differences in risk factor response from baseline to week 52 between the intervention and control groups. For each variable, differences in response between men and women were assessed by two-factor repeated-measures ANOVA using a Bonferroni adjustment. As above, t-tests compared baseline to week 52 changes between groups, by gender. To examine the potential confounding effects of medications on insulin and leptin response, sub-group analyses were conducted that excluded participants who changed the brand or dosage of any medication known to affect insulin, leptin, and/or lipid levels through (1) main (intended) effects or secondary (side) effects, or (2) main effects only.

Results

Baseline measures

At baseline, participants showed higher plasma insulin, % carbohydrate intake, BMI, and triglycerides, but lower % fat consumption and exercise capacity than controls despite the prospective matching strategy (Table 1). Insulin values did

Table 1 Cardiometabolic risk factors, dietary components, and physiological measures at baseline for participants and controls in the cardiac lifestyle program.

Measures	n	Controls	Participants	P[a]
Cardiometabolic risk factors				
Insulin (μU/ml)	150	14.3 ± 7.1	18.1 ± 10.2	0.012[b]
Leptin (ng/ml)	152	19.0 ± 17.2	23.5 ± 18.6	0.059[b]
Dietary components				
Calories (kcal/day)	114	1736 ± 582	2095 ± 776	0.056
% Carbohydrate intake	114	49.5 ± 9.2	54.0 ± 12.2	0.010[b]
% Fat intake	114	32.5 ± 8.5	28.8 ± 10.2	0.037
% Protein intake	114	16.7 ± 3.9	16.8 ± 6.3	0.591[b]
Physiological measures				
Age (years)	152	60.6 ± 7.6	60.6 ± 7.6	0.992
BMI (kg/m^2)	152	28.5 ± 4.5	32.9 ± 7.2	<0.001
Systolic BP (mm Hg)	146	132.0 ± 16.3	136.2 ± 16.9	0.119[b]
Diastolic BP (mm Hg)	146	78.6 ± 10.1	81.0 ± 10.1	0.238[b]
HDL cholesterol (mg/dl)	152	49.4 ± 13.0	45.5 ± 13.4	0.057
LDL cholesterol (mg/dl)	142	108.1 ± 33.8	112.5 ± 39.1	0.590
Total cholesterol (mg/dl)	152	185.3 ± 42.7	194.8 ± 48.2	0.200
Triglycerides (mg/dl)	152	143.5 ± 97.8	175.9 ± 94.2	0.004
Exercise capacity (Bruce score)	122	9.3 ± 2.9	6.6 ± 2.1	<0.001

Data are presented as mean ± SD; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] Tested by 1-factor ANOVA by cohort type.

[b] Tested by a nonparametric Mann–Whitney U test because data was not normally distributed after natural log transformation.

not differ significantly between participants who completed the program (graduates) and those who dropped out; however, leptin levels were higher (30.0 ± 18.4 versus 23.5 ± 18.6, $p < 0.05$) among dropouts. Dropouts also tended to be younger (55.1 ± 11.0 versus 60.6 ± 7.6, $p < 0.01$) and have lower systolic BP (130.4 ± 18.7 versus 136.1 ± 16.7, $p < 0.05$) than graduates. None of the risk factors differed between participants excluded from the analysis due to non-matching and those included in the study.

Changes in cardiometabolic risk factors

Participants in the cardiovascular health program experienced significant beneficial changes in plasma insulin and leptin (Table 2). Insulin levels declined ~19% in participants ($p < 0.001$ versus controls), while leptin levels decreased 33% ($p < 0.001$ versus controls). In contrast, both insulin (+4%) and leptin (+6%) increased in controls over one year.

Changes in dietary composition

Controls showed no significant change in any dietary component; whereas, participants reduced total caloric intake from >2000 calories/day to ~1700 calories/day (-18%) ($p < 0.05$ versus controls). Similarly, participants lowered daily fat intake by >60% ($p < 0.001$ versus controls) and, on average, maintained a total fat intake of ~11% of calories (Table 2). Carbohydrate intake increased by >30% among participants ($p < 0.001$ versus controls), while dietary protein remained unchanged.

Response of traditional CVD risk factors

Participants achieved a 9% reduction in BMI by the end of the year ($p < 0.001$ versus controls), a 6% reduction in diastolic BP ($p < 0.05$), and a 37% increase in physical fitness ($p < 0.001$), all significant improvements. Systolic BP and total cholesterol improved significantly from baseline to 52 weeks, but the degree of change did not differ between participants and controls. HDL decreased significantly by the end of the year, but overall change was not significantly different from controls.

Gender differences in response

Gender was not a significant factor for changes in any CVD risk factor from baseline to 52 weeks among controls. Men and women participating in the program showed similar significant improvement for insulin and leptin, nearly identical changes in diet (Fig. 1), and equivalent changes in BMI and physical fitness ($p < 0.001$) compared to controls after one year. Response for diastolic BP also was similar between genders — significantly different from baseline at 12 weeks and 52 weeks in participants, but the magnitude of change was not significantly different from controls. Triglyceride levels dropped significantly (−20%) among male participants ($p < 0.05$ versus controls), but in women, triglyceride response did not differ between participants and controls, and actually increased ~5% among female participants from baseline to the end of the year.

Table 2 Cardiometabolic risk factors, dietary components, and physiological measures for participants and controls in the cardiac lifestyle program at baseline, 12 weeks, and 52 weeks.

Measures	Controls ($n = 76$)				Participants ($n = 76$)				Between group P^a
	Baseline	Week 12	Week 52	% Change	Baseline	Week 12	Week 52	% Change	
Cardiometabolic risk factors									
Insulin	14.3 ± 7.1	14.9 ± 6.3	14.9 ± 6.8	+4.0	18.1 ± 10.2	$14.8 \pm 7.1^{**}$	$14.6 \pm 7.8^{***}$	−19.2	<0.001
Leptin	19.0 ± 17.2	16.5 ± 14.7	20.3 ± 16.8	+6.6	23.5 ± 18.6	$14.3 \pm 11.1^{***}$	$15.8 \pm 13.6^{***}$	−32.9	<0.001
Dietary components									
Calories	1736 ± 582	1736 ± 604	1633 ± 493	−5.9	2095 ± 776	$1545 \pm 333^{***}$	$1709 \pm 497^{***}$	−18.5	0.028
% Carbs	49.5 ± 9.2	49.0 ± 7.0	49.4 ± 8.9	−0.3	54.0 ± 12.2	$71.3 \pm 3.5^{***}$	$71.5 \pm 3.2^{***}$	+32.4	<0.001
% Fat	32.5 ± 8.5	33.2 ± 6.6	32.4 ± 7.2	−0.1	28.8 ± 10.2	$11.2 \pm 2.0^{***}$	$11.4 \pm 2.8^{***}$	−60.3	<0.001
% Protein	16.7 ± 3.9	16.5 ± 4.0	17.1 ± 4.6	+2.4	16.8 ± 6.3	17.3 ± 2.5	16.5 ± 2.4	−1.7	0.501
Physiological measures									
BMI	28.5 ± 4.5	28.3 ± 4.7	28.7 ± 4.8	+0.9	32.9 ± 7.2	$30.5 \pm 6.6^{***}$	$29.8 \pm 6.8^{***}$	−9.3	<0.001
SBP	132 ± 16	$126 \pm 15^{**}$	$125 \pm 13^{**}$	−5.3	136 ± 17	$122 \pm 14^{***}$	$127 \pm 17^{***}$	−6.4	0.562
DBP	78.6 ± 10.1	77.1 ± 8.3	77.3 ± 9.3	−1.5	81.0 ± 10.1	$73.0 \pm 9.0^{***}$	$75.5 \pm 9.5^{***}$	−6.7	0.022
HDL	49.4 ± 13.0	$52.0 \pm 13.1^{**}$	47.9 ± 13.3	−3.0	45.5 ± 13.4	$38.5 \pm 9.5^{***}$	$43.1 \pm 10.5^{*}$	−5.2	0.497
LDL	108 ± 34	106 ± 35	108 ± 34	−0.4	112 ± 39	$98 \pm 32^{***}$	109 ± 33	−2.8	0.536
TCH	185 ± 43	187 ± 46	185 ± 43	−0.2	195 ± 48	$170 \pm 43^{***}$	$185 \pm 44^{**}$	−5.0	0.066
TG	144 ± 98	156 ± 138	146 ± 88	+2.0	176 ± 94	163 ± 73	163 ± 93	−7.2	0.213
EC	9.3 ± 2.9	9.5 ± 2.8	9.3 ± 2.7	−0.6	6.6 ± 2.1	$8.4 \pm 2.2^{***}$	$9.0 \pm 2.6^{***}$	+37.6	<0.001

Data are presented as mean ± SD; % change is from baseline to week 52; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to baseline by repeated-measures ANOVA; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity.

[a] From independent samples *t*-tests (two-tailed) of baseline to week 52 changes in program participants compared to controls.

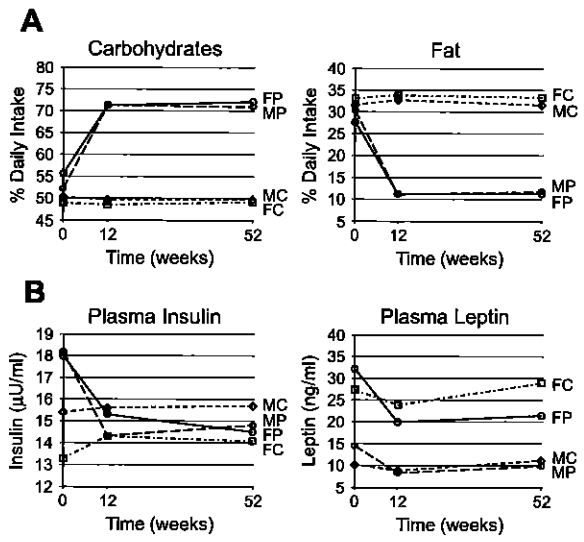

Figure 1 Changes in dietary composition (panel A) and cardiometabolic risk factors (panel B) among men and women participating in a year-long cardiovascular health program. FP, female participants; MP, male participants; FC, female controls; MC, male controls.

Effects of medications

Review of patient medical charts identified 114 prescription medications used by participants and controls at baseline. Medications ($n = 67$) known to influence circulating levels of insulin, leptin, and/or lipids as the primary intended effect, or as a secondary effect, were then partitioned into 8 categories based on function (Table 3). Separate analyses were used to assess the effects of (1) all medications in these 8 categories (composite medications), and (2) only medications influencing insulin, leptin, and lipids as a primary effect (primary medications). Because no medications were deemed to alter leptin or HDL as a primary effect, medications with the strongest secondary effects on these variables were examined.

Results of the sub-group analyses showed that composite and primary medications did not have significant effects on biomarker responses to the lifestyle change program (Table 4). Changes in insulin and leptin in participants and controls not taking medications known to influence these biomarkers or whose medication levels did not change during the study were similar to analyses encompassing all participants. Response for lipids was attenuated slightly when the effects of medications were considered. The largest effect was evident among controls, where LDL, total cholesterol, and triglyceride levels increased more in subjects with no medication changes.

Discussion

Participants who completed the year-long lifestyle change program reduced total caloric intake from >2000 calories/day to ~1700 calories/day, increased carbohydrate consumption by 30%, and decreased daily fat intake by 60%. The lifestyle intervention improved circulating levels of insulin (−19%) and leptin (−33%), which contribute to cardiometabolic risk, as well as traditional cardiovascular risk factors. Changes in circulating insulin and leptin were comparable to, or superior to, responses reported in other dietary or exercise interventions (Web Appendix), and were not significantly influenced by medication use. Men and women showed similar beneficial changes for most risk factors.

The term "cardiometabolic risk" for developing coronary atherosclerosis encompasses risk factors such as age, gender, high cholesterol, hypertension, smoking, and obesity plus additional contributing factors including insulin resistance, vascular inflammation, atherogenic dyslipidemia, and poor lifestyle behaviors. Leptin may contribute to

Table 3 Medications used by participants and controls in the cardiac lifestyle program known to affect plasma levels of insulin, leptin, and lipids.

Medication Category (n)[a]	Insulin	Leptin	HDL	LDL	TCH	TG
ACE inhibitors (13)	↓	↓	↑ ns	↓ ns	↓ ns	↓
Anticoagulants (2)						
Platelet aggregation inhibitors					↑	
Beta blockers (10)	↑	↑	↓	↓ ns	↑ ns	↑
Calcium channel blockers (9)	↑−	↓	↑−	↑−	↑−	↓−
Insulin medications (3)	▲[b]	↑[b*]				
Diuretics (6)			↓ ns	↑ ns	↑	↑
Lipid lowering medications (16)			↑[b]	▼[b]	▼[b]	▼[b]
Oral antihyperglycemics (8)						
Thiazolidinediones			↓	↑	↑	↑
Biguanides	↓	↓	↑	↓	↓	↓
Sulfonylureas	↑					

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; ACE, angiotensin converting enzyme; ns, not statistically significant; −, effect may be neutral. Adapted from [29].
Key to medication effects: ▲ − increase, main effect; ↑ − increase, secondary effect; ▼ − decrease, main effect; ↓ − decrease, secondary effect, * − dose dependent.
[a] The number of brand name medications in each category is indicated in parentheses.
[b] Considered a primary medication in Table 4.

Table 4 Effects of medication changes on cardiometabolic risk factors and lipid measures for participants and controls in the cardiac lifestyle program.

Measures	All participants			Composite medications[a]			Primary medications[b]		
	% Change at week 52 (*n*)		Between group P^c	% Change at week 52 (*n*)		Between group P^c	% Change at week 52 (*n*)		Between group P^c
	Controls	Participants		Controls	Participants		Controls	Participants	
Cardiometabolic risk factors									
Insulin	+4.0 (75)	−19.2*** (76)	<0.001	+3.0 (61)	−16.0** (54)	0.005	+4.4 (74)	−20.3*** (75)	<0.001
Leptin	+6.6 (76)	−32.9*** (76)	<0.001	+7.9 (61)	−32.7*** (48)	<0.001	+9.1* (75)	−33.6*** (75)	<0.001
Lipid measures									
HDL	−3.0 (76)	−5.2* (76)	0.497	−2.7 (45)	−6.0* (54)	0.372	−2.8 (50)	−5.9* (60)	0.375
LDL	−0.4 (71)	−2.8 (71)	0.536	+4.3 (49)	−0.2 (58)	0.230	+4.3 (49)	−0.2 (58)	0.230
TCH	−0.2 (76)	−5.0** (76)	0.066	+2.6 (42)	−2.0 (44)	0.126	+3.1 (50)	−3.4* (60)	0.013
TG	+2.0 (76)	−7.2 (76)	0.213	+4.5 (44)	+0.8 (43)	0.798	+7.3 (50)	−4.9 (60)	0.214

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to baseline by repeated-measures ANOVA; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides.

[a] Composite medication categories are described in Table 3.

[b] Primary medication categories include: insulin and leptin — insulin medications; HDL, LDL, TCH, and TG — lipid lowering medications.

[c] From independent samples *t*-tests (two-tailed) of baseline to week 52 changes in intervention participants compared to controls.

cardiometabolic risk through atherogenic effects on the vasculature, stimulating production of reactive oxygen species and proinflammatory cytokines, which leads to oxidative stress, vascular inflammation, and atherosclerotic lesion formation [19]. Similarly, insulin stimulates the actions of various growth factors within the vasculature leading to inflammation and endothelial dysfunction [20].

Lifestyle modification can delay or prevent progression of atherosclerotic disease and significantly reduce risk of CVD mortality [21,22]. Therefore, interventions that modify both cardiovascular and metabolic risk factors may have the greatest potential to mediate cardiometabolic risk. In this study, participants in a cardiovascular lifestyle program showed significant reductions in plasma insulin and leptin, which may have beneficial anti-inflammatory and anti-oxidative effects on the vasculature.

Despite evidence that lifestyle modification can lead to significant improvements in overall cardiovascular risk profiles, gender differences in response of plasma insulin and leptin to exercise training [23] and a combination of diet and physical activity [24] have been reported. Men and women in our program showed similar reductions in plasma insulin and leptin, likely caused by changes in dietary composition and increased physical activity. These behaviors resulted in significant weight loss in both men and women over one year. Although gender differences may exist in the physiological action of insulin and leptin within the vasculature, fasting insulin and leptin are strongly correlated with percent body fat. Thus, through diet, exercise, and weight loss, both men and women may have derived similar benefit in terms of cardiometabolic risk reduction.

High consumption of fruits, vegetables, and whole grains has been associated with a favorable CVD biomarker profile, including lower fasting insulin and leptin concentrations [25]. Likewise, physical activity sustained for at least four weeks has a meaningful effect on insulin, leptin, and several other blood biomarkers implicated in CVD [26]. At baseline, program participants and controls consumed a high fat diet normally associated with obesity, insulin resistance, and atherosclerosis. Participants in the lifestyle program successfully transitioned to a low-fat diet and dramatically increased their level of physical activity, which may have been important for reducing plasma insulin and leptin levels.

One incidental benefit experienced by some participants in the cardiac lifestyle program is a reduction in the number and/or dosage of prescription medications, which has the potential to influence changes in metabolic and cardiovascular risk factors. To remove the influence of medications on risk factor response during the program, we conducted a sub-group analysis that excluded participants who changed the brand or dosage of any medication known to affect insulin, leptin, and/or lipid levels. These analyses indicate that medications did not have significant effects on biomarker response and suggest that changes in cardiometabolic risk factors during the program are primarily attributable to lifestyle changes.

Strengths and limitations

Cardiometabolic risk factors are rarely examined simultaneously in cardiac lifestyle modification programs with validated protocols and data collection methods. The prospective, longitudinal nature of this study and availability of matched controls minimized sources of bias and confounding and improved our ability to assess treatment benefits. Participants remained under the care of their primary physician, who may have prescribed changes in medications affecting plasma insulin or leptin levels. Removing pharmacological influences on risk factor modification strengthened the conclusion that participants derived meaningful metabolic and cardiovascular benefit from the program.

The Ornish Program is an established treatment alternative for CVD patients involving demanding lifestyle changes that requires motivation and significant time commitment. Baseline differences between cases and matched controls indicate that participants have

particularly atherogenic CVD risk factor profiles and would benefit most from cardiovascular risk reduction. Because careful screening is essential to identify motivated patients who would adhere to program guidelines, it was impractical to use a randomized study design. However, well-designed case–control studies are highly similar to randomized trials for estimating treatment effects [27,28]. We analyzed the data using a per-protocol approach, which included only patients who completed the program, rather than an intent-to-treat analysis. The lifestyle intervention included multiple modalities over one year, thus we were able to evaluate only short-term changes in cardiometabolic risk factors and were not able to define the relative contribution of each program component. Further, we could not assess applicability to the general public and whether results observed here are achievable outside of a controlled clinical environment. Future research will determine if improvements in cardiometabolic risk continue after participation and translate into improved clinical outcomes and develop less-rigorous cardiac interventions to maximize adherence and cardiovascular/metabolic benefit.

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Authors' contribution

LV drafted the paper and performed dietary analysis, DN managed the patient database and performed statistical analysis; DD partitioned name brand medications into functional categories, defined primary and secondary effects, and determined comparable dosages; AB and MH conducted the lifestyle intervention; FL collected dietary data and directed dietary analysis; HP collected and processed blood samples, coordinated biomarker assays; MV reviewed the paper and provided oversight as PI of ICHP; DE conceived and supervised the study and drafted the paper, which was reviewed critically by all authors.

Competing interests

The authors report no conflicts of interest with this study.

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Appendix. Supplementary data

Supplementary data related to this article can be found in the online version at doi:10.1016/j.numecd.2012.01.012.

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ORIGINAL RESEARCH

Stress Therapy Empowering Prevention (STEP): A Healthy Lifestyle Program for Breast Cancer Patients

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Purpose: Develop and implement a comprehensive program for lifestyle change, empowering breast cancer patients to manage stress effectively and improve their mental and physical health.
Method: Women with breast disease (or those at high risk) are offered a program of lifestyle change, consisting of a Healthy Lifestyle intervention for 3 months followed by monthly contact with a health coach. Instruction and demonstration provide information on exercise, nutrition, stress reduction, and mind/body health. Examinations are conducted at baseline, after completion of the intervention (3 months), at 1 year, and every 6 months for a period of 5 years.
Conclusion: Breast cancer has a significant emotional, psychological, and social impact and is often associated with high levels of stress that promote unhealthy behaviors causing weight gain, decreased physical fitness, and an increased risk for cardiovascular disease (CVD). Similar to CVD, research shows breast cancer susceptibility is also influenced in part by modifiable risk factors, suggesting that a healthy lifestyle program may lead to reductions in cancer risk and recurrence as well as improvements in mental health and quality of life. Through the Stress Therapy Empowering Prevention (STEP) program, breast cancer and high-risk patients are empowered with tools to focus on health promotion and optimization and maintenance of quality of life. Patients can improve physical and psychosocial factors in as little as 3 months, but long-term follow-up will determine if lifestyle changes result in improved clinical outcomes over time.

Extensive reports have documented the relationship between lifestyle changes and morbidity/mortality associated with cardiovascular disease (CVD). In particular, diet, physical activity, and stress are known to be associated with cardiovascular morbidity and mortality.[1-3] Similar to CVD, evidence has been mounting that breast cancer susceptibility is influenced in part by modifiable risk factors, such as body weight, diet, and physical activity, suggesting that a healthy lifestyle program may lead to a reduction in risk factors for CVD and breast cancer. Improving health and quality of life in patients with CVD and breast cancer will result in improved outcomes of care over the long term.

Early diagnosis and treatment are still vital to surviving breast cancer. Although an estimated 192,370 new cases of invasive breast cancer were expected in 2009, with approximately 40,170 deaths from the disease, incidence rates actually decreased by 2.0% per year,[4] likely due to advanced screening and early detection. In an effort to continue to lower incidence rates and improve long-term outcomes, studies of behavior modification in breast cancer patients are providing new information about how lifestyle factors affect survivorship as well as knowledge to help develop new, effective intervention programs to decrease breast cancer risk.[5-11]

The Stress Therapy Empowering Prevention (STEP) program is an innovative approach based on the concept that comprehensive lifestyle changes may have a meaningful impact on the risk for developing breast and cardiac disease. Given the advantages of a healthy lifestyle on both physical and emotional outcomes, cancer patients as well as those at high risk should be urged to address unhealthy behaviors. Our STEP model utilizes a specialized team comprising physicians, nurses, dietitians, licensed therapists, exercise physiologists, and stress management specialists who provide comprehensive strategies

that empower the participant to make healthier choices at an individual level. The program is an adjunct to treatment and care that participants receive from their personal healthcare providers. This combined effort allows for closer monitoring of each participant and coordination of care across the healthcare spectrum to achieve optimal health and quality of life.

METHODS

The overall goal is to recruit and evaluate approximately 500 women diagnosed with, or at high risk for, both breast and cardiac disease. The objectives of the study are to 1) test the efficacy of a healthy lifestyle intervention on reducing stress, sleep disturbances, and cardiovascular risk factors in both high-risk patients and patients diagnosed with breast disease; 2) evaluate the long-term benefit of an enhanced health coach intervention in promoting sustained wellness behaviors; and 3) examine molecular markers common to atherosclerosis and cancer to assess longitudinal changes and their relationship to disease development.

The STEP program has a 3-month healthy lifestyle intervention period during which participants meet once a week to learn the program guidelines, which include a low-fat, whole food nutrition plan based on the Mediterranean diet, aerobic and strength training exercises, stress management, and weekly mind/body health sessions. After the initial 3-month period, participants are contacted on a monthly basis by a health coach to ensure that program compliance is being maintained and to assist with long-term adherence. Participants are required to return to the center at the 1-year time point, and every 6 months thereafter for a period of 5 years, for testing and evaluation. Information collected includes perceived stress, sleep disturbance, psychosocial measurements, carotid ultrasound to measure carotid intima-media thickness, traditional risk factors (weight, blood pressure, body mass index [BMI], body composition), and biochemical assays.

To be eligible to participate, women must be 18 years of age or older with a diagnosis of breast disease (atypical hyperplasia, in situ carcinoma, or invasive breast cancer) or significant risk factors for developing breast disease such as previous biopsy, family history of breast disease, first pregnancy after the age of 30, early menstruation or late onset of menopause, or high risk of developing coronary artery disease (CAD) as indicated by having 1 or more of the following: family history of CAD, hypertension, diabetes, smoking, elevated blood lipids, sedentary lifestyle and obesity, or established clinically stable coronary disease.

Participants begin the program with an extensive physician visit to conduct a comprehensive risk assessment and develop a realistic lifestyle change plan. Participants are interviewed to assess sleep patterns, smoking status, cardiovascular and breast history, and medication use. The clinical exam includes height and weight measurements to calculate BMI (kg/m^2); blood profiles including thyroid-stimulating hormone, comprehensive metabolic panel, and fasting glucose and lipid panel; systolic and diastolic blood pressures; and psychological screening to evaluate mental health. Assessments are repeated at the end of the Healthy Intervention, at year 1, and every 6 months thereafter for a period of 5 years.

> "Our STEP model utilizes a specialized team comprising physicians, nurses, dietitians, licensed therapists, exercise physiologists, and stress management specialists..."

Following the initial examinations, participants attend an educational workshop designed to provide further instruction regarding the recommended lifestyle changes, followed by once-a-week sessions over a 3-month period. These sessions are tailored to ensure that each individual receives the appropriate education and experience needed to achieve success. Participants are required to complete a personal awareness log each week, which includes documentation of diet, exercise, and stress management frequency and duration, and a self-report of their mind/body session experience.

Blood samples are obtained from each consenting individual at baseline, at completion of the healthy lifestyle intervention, at 1 year, and every 6 months thereafter for a period of 5 years. From the blood samples, the following biochemical assays are analyzed: 1) lipoprotein subclass distributions determined by nuclear magnetic reso-

nance (NMR) spectroscopy; 2) stress/CVD biomarker panel: serum cortisol, insulin, leptin, highly sensitive C-reactive protein, lipoprotein(a), adiponectin, resistin, serum amyloid A, and vitamin D; and 3) breast disease–related panel: HER2/*neu*, tumor necrosis factor (TNF) alpha, and estradiol. In addition, blood is collected for isolating messenger RNA to determine changes in gene expression over the course of the study and identify new molecular markers associated with improved CVD biomarker risk profiles.

"Upon completion of the healthy lifestyle intervention (3 months), participants (n = 14) showed change in the desired direction for many risk factors."

RESULTS

Recruitment is being conducted primarily through newspaper and radio ads; distribution of patient information brochures; and speaking engagements at various community education events, physician offices, and support groups. Of 43 women who initially expressed interest in the program, 18 have enrolled thus far. Average age of participants was 65 years. Of the 18 participants enrolled in the program, 11 women had diagnosed breast disease (61%). In addition, of these same 18 women, 17 (94%) were also considered at high risk for developing CVD by having at least 1 documented CAD risk factor. Overall attendance was 88% during the initial 3-month on-site sessions. Four participants (22%) discontinued participation in the program, 3 due to personal, nonmedical reasons, and 1 due to breast cancer progression.

Upon completion of the healthy lifestyle intervention (3 months), participants (n = 14) showed change in the desired direction for many risk factors. Body weight (-1.8%, P <.05), BMI (-2.5%, P <.05), and perceived stress (-22.1%, P <.05) decreased significantly. Diastolic blood pressure (-8.4%, P <.08) and sleep quality (-26.5%, P <.06) showed near-significant changes. Most importantly, at the 1-year time point, perceived stress (n = 10, 8.2%, P <.05) and sleep quality (n = 9, -4.9%, P < .05) improvements were maintained, showing that these positive changes could be maintained over a longer period of time. In addition, though lacking statistical significance with our current sample size, triglycerides, systolic blood pressure, hostility, and depression all decreased at both time points (**Table**).

Based on self-reported exercise frequency and duration data, at 3 months participants on average were able to increase vigorous activity (heavy lifting, digging, aerobics, or fast bicycling) by 1.13 days/week, moderate activity requiring the participant to breathe somewhat harder than normal (carrying light loads or bicycling at a regular pace) by 1.56 days/week, and walking activity (including walking at work or home for recreation, sport, exercise, or leisure) by 1.63 days/week. At the 1-year time point, participants continued to show increased levels of activity for all measured categories; vigorous activity remained increased by 1.13 days/week, moderate activity by 1.13 days, and walking activity by 0.82 days when compared with baseline activity.

Lipoprotein subclass profiles will be assessed by NMR spectroscopy, which will quantify low-density lipoprotein particle number and size, and provide direct measurement of high-density lipoprotein and very low-density lipoprotein subclasses. Biochemical variables of interest regarding CVD risk, including insulin, leptin, lipoprotein(a), adiponectin, resistin, serum amyloid A, and TNF alpha will permit correlation of traditional CVD risk factors with nontraditional biomarkers to provide more information on the prevention and treatment of CVD. Vitamin D, HER2-*neu*, and estradiol will be analyzed to provide further insight into breast disease development and progression. Lower serum 25 (OH) D (vitamin D) concentrations may be associated with poorer overall survival and distant disease-free survival in postmenopausal breast cancer patients.[12] HER2-*neu* blood levels have potential as a tumor marker in breast cancer. Many studies have monitored circulating levels after surgery and reported that increasing HER2-*neu* levels can indicate recurrence of breast cancer earlier than clinical diagnosis.[13,14] Estrogens are believed to play a critical role in the etiology of breast cancer, and considerable evidence suggests that lifetime exposure to endogenous hormones, notably estrogens, promotes breast carcinogenesis.[15] Finally, cortisol levels, considered a major indicator of altered psychological states in response to stress, may provide information on short- and long-term stressors.[16]

Table Change in Selected Physical and Psychosocial Variables

Outcome	Baseline	3 Months	%Δ	*P**	1 Year	%Δ	*P**
Weight	180.5	177.3	-1.8	<.05	175.6	-2.7	.10
Body mass index	32.5	31.7	-2.5	<.05	31.5	-3.1	.38
Total cholesterol	201.1	200.2	-0.5	.90	203.4	+1.1	.73
Triglycerides	157.2	136.7	-13.0	.17	147.6	-6.1	.52
Systolic blood pressure	134.0	125.1	-6.6	.15	126.4	-5.7	.23
Diastolic blood pressure	79.6	72.9	-8.4	.08	76.3	-1.3	.28
Glycosylated Hgb	6.4	6.6	+3.1	.34	6.2	-3.1	.30
Fasting glucose	108.6	110.6	+1.8	.75	112.4	+3.5	.51
Depression	13.7	11.3	-17.5	.23	8.5	-38.0	.11
Hostility	6.4	4.8	-25.0	.16	5.7	-10.9	.33
Perceived stress	16.3	12.7	-22.1	<.05	11.7	-28.2	<.05
Pittsburgh sleep quality index	10.2	7.5	-26.5	.06	9.7	-4.9	<.05

*P value based on repeated-measures analysis of variance.

DISCUSSION

There are no proven substitutes for conventional cancer treatments such as surgery, chemotherapy, radiation, and immunotherapy; however, one approach to gaining a better understanding of how lifestyle change can enhance breast cancer survival is to develop studies that address several behavior and lifestyle factors within the same program. Research has shown that among women with breast cancer who had surgery and conventional treatment, those who learned to change their lifestyle through education focused on better nutrition, more exercise, and stress reduction were 68% less likely to die from disease over an 11-year period than those who did not.[17] Although the STEP study currently lacks long-term follow-up data, our program is examining the importance of helping breast cancer patients eat better, lose weight, improve strength and endurance, develop coping skills, and ultimately to improve their overall health and well-being. Participants in a STEP-style program feel better, both physically and emotionally. These observations suggest that the program has potential to improve their long-term overall risk profiles.

An important finding in our study was the struggle encountered in recruiting participants into the program. Obstacles to recruitment included out-of-pocket expenses, lack of local physician referrals, participant time constraints, and lack of knowledge among patients about the benefits of lifestyle change on quality of life or clinical outcomes. However, once women made the commitment to participate, surveys indicated a high degree of satisfaction with the program. Ultimately, issues encountered with recruitment affected our sample size, leading to difficulties in being able to effectively interpret preliminary data. In the future, we will continue to use best clinical judgment on when to approach appropriate patients based on past experience, to repeatedly offer to assist patients with addressing risk factors, and to educate healthcare providers about the STEP program to increase our sample size and provide additional data for analysis of the effects of lifestyle change on breast disease.

NUTRITION

Although the relationship between diet and breast cancer remains unclear, studies have shown that improved nutrition reduces the risk of several chronic diseases, such as obesity, diabetes, and heart disease, and that a healthy lifestyle improves overall quality of life.[18,19] Breast cancer patients who practice better nutrition are likely to derive benefit in terms of total mortality, similar to the general population. The Women's Healthy Eating and Living study showed that women who consumed a healthy diet and were physically active increased survival after diagnosis.[20] Patients who reported eating at least 5 servings of fruits and vegetables per day and performing 30 minutes of moderate walking 6 days a week reduced the probability of death by 50%.

The STEP program nutrition plan is based on

the Mediterranean diet and recommends eating vegetables; fruits; whole grains; lean protein sources such as fish, nuts, and olive oil; and minimizing the amount of red meat consumed. Participants are counseled to focus on eating more naturally occurring and fewer highly processed foods. Involvement of a registered dietitian helps to guide this process and provides the education, support, and long-term follow-up needed to meet the challenges of sustaining the recommended dietary changes.

The majority of studies of diet and breast cancer have examined the impact of body weight on survival. Most have observed that obesity at diagnosis is associated with poor prognosis.[21] Similarly, weight gain after diagnosis is common and is associated with mortality, disease recurrence, and development of comorbid conditions including diabetes and CVD.[22] Although some studies have shown that following a prudent diet alone, without adding physical activity, may not be associated with breast cancer survival,[5,23] a healthy diet has been shown to have beneficial effects on overall survival in conditions such as diabetes and heart disease, which are frequently seen in breast cancer patients.[24]

Participants in the STEP program were able to significantly decrease measures of obesity such as weight and BMI within the first 3 months of the program. Although these measures were not statistically significant at 1 year, they continue to remain lower than at baseline, suggesting that participants were successful in meeting or exceeding dietary compliance targets, thus preventing weight gain and promoting weight loss, which has been proven to be an effective strategy for improving overall quality of life and survival.

EXERCISE

Physical activity is as important as diet for achieving optimal weight and maintaining a healthy lifestyle. In studies examining the relationship between physical activity and the risk of breast cancer, a decrease in risk of approximately 25% was found among the most physically active women.[25] Similarly, in studies examining the effect of physical activity on breast cancer survival, some studies suggest that postdiagnosis physical activity may have great benefit. One study showed that after diagnosis, physical activity equivalent to walking 3 to 5 hours per week reduced mortality by as much as 50%.[26] Although the risk of developing comorbid conditions, including CVD, type 2 diabetes, fatigue, lymphedema, psychological distress, and poor quality of life, often persists in breast cancer survivors, recent studies have shown that physical activity can lower breast cancer risk and provide additional health benefits, such as decreased risk of stroke and type 2 diabetes, and improved longevity and quality of life.[27]

Most STEP participants achieved improvement in physical activity during the initial 3-month period, and many maintained these initial gains or continued to improve by the end of the first year. While most research demonstrates beneficial effects between physical activity and overall health, it is important to recognize that there is a risk-benefit ratio to exercise that may be different for each breast cancer patient. Utilizing a personalized plan might be most effective because it can be customized for different time periods, from prediagnosis through cancer treatment, based on individual needs and abilities. The STEP program develops each participant's activity plan based on an individual assessment completed by an exercise physiologist, but generally participants are encouraged to exercise aerobically for a minimum of 30 minutes per day, for a total of 3 hours of aerobic exercise each week. More intense exercise is permitted if medically appropriate and desired by the participant. Resistive or strength training exercise also is important, and if medically appropriate, participants were instructed to engage in strength training exercises 2 to 3 times per week. During the healthy lifestyle intervention portion of the study, hour-long supervised exercise sessions were scheduled.

The objectives of our exercise modality are to fully understand the importance and benefits of regular physical activity, to create a safe environment for exercise, and to encourage participants to properly monitor their own exercise program outside of the STEP program. These activities will assist with long-term adherence and allow the participant to achieve her own physical activity goals.

STRESS MANAGEMENT

Working with participants in the STEP program presents some unique challenges. These women have faced their mortality and live with the ongoing psychological stress of possible cancer recurrence.[28] A recent meta-analysis of 10 randomized controlled trials found that cancer patients who

participated in yoga interventions showed significant improvement in several psychological measures, including anxiety, distress, depression, and stress compared with wait-list controls.[29] For breast cancer survivors in particular, yoga has been shown to improve quality of life and emotional functioning.[30]

A mild form of physical activity, such as yoga or tai chi, may help to promote regular participation in physical activity. The therapeutic application of yoga enables participants to move slowly and safely, concentrating on relaxing their body while building flexibility, strength, and balance, which is especially important in breast cancer patients who may face additional barriers to more vigorous physical activity.[31] As emotional stress has been associated with decreased survival in breast cancer patients,[32] possibly by muting immune functions and accelerating the inflammatory response, stress management may offer a real survival advantage to cancer patients in addition to emotional benefits.

The STEP program's stress management specialist is a certified yoga therapist trained in techniques to provide participants with healthier ways to deal with the stress of living with a potentially life-threatening disease. The practice of yoga relies on physical postures to stretch muscles, focused breathing and meditation to minimize stress through visualization techniques, and guided imagery. Throughout the initial intervention, stress management sessions are held once a week. During these sessions, participants receive education and training in performing these techniques. The result is a relaxed body and a peaceful state of mind. Daily stress management practice was encouraged in the STEP program so that these techniques would be routine when patients are faced with a stressful situation.

MIND/BODY HEALTH

Women with breast cancer often exhibit emotional distress similar to posttraumatic stress disorder (PTSD).[33,34] In a recent study, among women who were recruited an average of 47 months following diagnosis of breast cancer, 38% had moderate to high anxiety, 22% had moderate to high depression, and PTSD was observed in 12%.[35] These findings show that the emotional impact of breast cancer can last for years following diagnosis. In addition, women lacking a social network had a significantly higher risk of breast cancer mortality than women with strong social ties to relatives, friends, and neighbors. Breast cancer patients often experience social isolation due to treatment, body image issues, or fatigue, which can have significant detrimental effects on psychological well-being by increasing levels of anxiety and depression. Therefore, it is important to recognize the signs of psychological distress in breast cancer patients and develop programs that effectively manage stress and mental health.[36]

The mind/body sessions in the STEP program are facilitated by a licensed therapist. These sessions are designed to create an atmosphere in which participants feel comfortable expressing their feelings and personal experiences. Since all STEP participants share common ground, individuals who self-disclose their experiences in dealing with breast disease encourage other participants to share their experiences as well. The overall purpose of the mind/body session is to create an environment where participants can experience belonging and the feeling of being connected. It is important to understand that these sessions are not group therapy—they are intended to facilitate making and sustaining healthy behaviors every day. Most of us know what we need to do to lead healthier lifestyles, but change is difficult to attain and sustain without ongoing support. This component upholds accountability, and the participants come to depend on each other for ongoing support.

CONCLUSION

In summary, lifestyle change interventions have proven to be beneficial to the vast majority of participants, but there are a limited number of studies that have examined the effect of combining several lifestyle behaviors into one comprehensive program to benefit breast cancer patients. The STEP program is a pioneer program that has combined the efforts of conventional treatment regimens with simple lifestyle changes, empowering breast cancer patients to actively manage their disease. As well-powered randomized controlled trials continue to define the effectiveness of lifestyle modification, hopefully more comprehensive programs will become available and eventually translate into improved care for breast cancer patients.

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Perceived stress correlates with disturbed sleep: A link connecting stress and cardiovascular disease

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Abstract

The association between stress and cardiovascular disease (CVD) risk is becoming established. A mechanistic link clarifying the intermediate steps between the experience of stress and the development of CVD would support this association. We sought to examine the role of perceived stress as a factor associated with disturbed sleep with the goal of providing an explanation for the stress–CVD connection. We performed a cross-sectional analysis of data recorded by subjects at entry to our CVD prevention program. Data collection included questionnaire surveys, anthropometrics, and a CVD-relevant laboratory panel. Of 350 consecutively enrolled subjects (mean age 54.4 ± 12.4 [SD] years, 138 men, 39%), 165 (47%) scored above the mean for stress measures. These high-stress subjects displayed an increased cardiovascular risk profile including elevated body mass index (mean ± SD 31.1 ± 5.9 vs. 29.0 ± 5.9, $r_s = 0.175$), increased waist circumference (102 ± 17 cm vs. 98 ± 14, $r_s = 0.135$), and elevated high-sensitivity serum C-reactive protein (0.384 mg/dl vs. 0.356, $r_s = 0.109$). High-stress subjects also demonstrated greater daytime sleepiness (Epworth Sleepiness Scale: 10.4 ± 5.0 vs. 7.8 ± 4.8, $r_s < 0.316$), greater fatigue (fatigue scale: 5.4 ± 2.2 vs. 3.4 ± 2.4, $r_s = 0.484$), poorer sleep quality (Pittsburgh Sleep Quality Index: 8.5 ± 4.4 vs. 5.9 ± 4.0, $r_s = 0.416$), and shorter sleep duration (20 min less/24 h, r_s = negative 0.177) with a higher risk for sleep apnea (60% at high risk vs. 40%, $p = 0.003$) than low-stress subjects. High stress was associated with significant disturbances in sleep duration and sleep quality. Stress levels also correlated with daytime consequences of disturbed sleep. The stress–sleep connection may be an important mechanistic mediator of the association between stress and CVD.

Keywords: *Cardiovascular disease, perceived stress, risk factors, sleep, sleep quality, stress*

Introduction

An emerging body of evidence substantiates the observation that there is an association between stress and the occurrence of cardiovascular disease (CVD; Belkic et al. 2004; Rosengren et al. 2004). While the stress–CVD connection has been promoted and taught for decades, a number of difficulties have slowed a productive line of investigation in this area.

The impediments include how to define and measure stress (Cohen et al. 1983; Kocalevent et al. 2007), how to assess stress levels in a reproducible fashion over time (Kocalevent et al. 2009), uncertainty regarding causation between stress and CVD (Tindle et al. 2010), and the expense of performing a study to follow large numbers of subjects over a substantial period of time (Kadojić et al. 1999; Hamer et al. 2008). Furthermore, some studies appear to contradict the association between stress and CVD (Greenlund et al. 1995; Riese et al. 2000; Heslop et al. 2002a,b; Belkic et al. 2004). Nevertheless, the preponderance of studies to date supports the conclusion that stress, variously defined in a variety of approaches, does correlate with increased cardiovascular risk, both for heart disease (Melamed et al. 1992; Belkic et al. 2004; Rosengren et al. 2004; Brborović et al. 2009; Holden et al. 2010; Puustinen et al. 2010) and stroke (Everson et al. 2001; Surtees et al. 2008; Tsutsumi et al. 2009). Moreover, studies of depressive behaviors in female primates subjected to social stressors over a 4-year period have demonstrated significant acceleration of coronary artery atherosclerosis, suggesting a causal relationship between stress and CVD (Shively et al. 2008).

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To substantiate and explain the clinical observations correlating stress and CVD, it would be useful to clarify the underlying pathophysiology to outline mechanisms that link stress and CVD. It is clear that the hypothalamus–pituitary–adrenal axis plays a major role, by stimulating cortisol secretion, as do increased aldosterone and catecholamine levels, with a resulting detrimental effect on the cardiovascular system (Kubzansky and Adler 2010). It remains less clear what maladaptive conditions initiate the cascade of mediators that trigger these responses.

One mechanism was proposed in the Massa Lombarda Project, an epidemiological study including 7000 northern Italian adults (Bove et al. 2010). In a subset of 106 men and women, selected for older age, psycho-emotional stress and depression disorder were associated with the development of metabolic syndrome, a cluster of multiple cardiovascular risk states. Another study examined whether self-reported job strain was associated with early, potentially modifiable cardiovascular (CVD)-related health behaviors (Hellerstedt and Jeffery 1997). This study of 3843 randomly selected men and women employees of 32 worksites in Minnesota showed that work stress, defined as high demand and low latitude, was positively associated with smoking, smoking intensity, and high fat intake in men, and with body mass index (BMI) and smoking intensity in women. In 2008, the most sophisticated studies to date were published to describe the mechanistic links between stress and CVD (Chandola et al. 2008; Hamer et al. 2008). These studies used statistical models to assess the relative contributions of potential mediators of stress and CVD events. The Whitehall II study followed over 10,000 male and female civil servants for an average of 12 years (Chandola et al. 2008). The study showed that two factors, health behaviors and metabolic syndrome, accounted for around 32% of the effect of work stress on CVD. Another study that used statistical modeling was prospective and included 6576 healthy men and women followed over an average of 7.2 years (Hamer et al. 2008). Psychological distress was measured with the validated General Health Questionnaire, and actual CVD events (hospitalization for nonfatal myocardial infarction, coronary artery bypass, angioplasty, stroke, heart failure, and CVD-related mortality) were used as the main outcome. The investigators reported that behavioral factors explained the largest proportion of variance (approximately 65%), whereas pathophysiological factors accounted for a modest amount (C-reactive protein approximately 5.5%; hypertension approximately 13%).

The mechanisms proposed by these studies, while supported by objective data, fail to fully account for the observed relationship between stress and CVD. An often overlooked contributor to ill health and bad medical outcomes is sleep, with important sleep parameters including sleep duration and sleep quality. Failure to include the role of sleep in the stress–CVD connection is especially surprising in view of the substantial personal experience that all humans have of the ill effects of sleep deprivation and disrupted quality of sleep. Understanding the role of sleep as a possible link between stress and CVD is especially appealing because sleep behaviors can be taught and improved. Furthermore, it has been shown that improving sleep quality through the implementation of behavior modification does lower perceived stress levels (Eliasson et al. 2010). Disrupted sleep is thus a modifiable risk factor for stress levels and may therefore be, in extension, a modifiable risk factor for CVD.

We therefore hypothesized that high levels of perceived stress would correlate with disturbed sleep parameters. Such mechanistic link is indicated by substantial prior research showing that short sleep and disrupted sleep are associated with high risk for CVD (Heslop et al. 2002a,b).

Methods

The investigation was conducted with the approval of our institutional review board. The study design is a retrospective analysis of data collected on consecutive patients participating in a CVD prevention program at the Walter Reed Army Medical Center Integrative Cardiac Health Project (ICHP). ICHP is a cardiovascular prevention research center for the US Department of Defense. All data were retrospectively gathered and no blood samples were taken specifically for this study. The institutional review board, therefore, did not request informed consent from the study subjects.

Patients were self-referred or referred to the program by a health-care provider to improve habits of diet, exercise, sleep, and stress management. ICHP is accessible to military health-care beneficiaries including active duty service members, retirees, and dependents. The program, therefore, enrolls a broad spectrum of subjects including a variety of races and ethnic backgrounds, both genders, and a range of ages from 18 to 90 years. The typical patient entering the program is found to have two to four risk factors for CVD.

Upon entry, subjects are asked to complete a series of questionnaires to gather information on demographics, current symptoms, past and current medical conditions including medications and lifestyle habits. Among the questionnaires are the validated surveys to assess stress levels, sleep behaviors, sleep quality, and daytime symptoms from inadequate sleep. Data from the questionnaires are reviewed during a medical interview with a nurse practitioner who also performs a physical examination to include anthropomorphic measures.

Laboratory studies

Subjects gave blood for cardiac-relevant biochemical studies. For all blood samples, subjects were instructed to present to the laboratory between 06:00 and 08:00 h having fasted from 20:00 h the previous evening. The biochemical measurements on blood samples included a standard lipid panel with total cholesterol concentration, low-density lipoprotein (LDL) cholesterol concentration, high-density lipoprotein (HDL) cholesterol concentration, triglyceride concentration, as well as lipoprotein (a) and lipoprotein PLA2 concentrations. Measures of glucose metabolism include fasting plasma glucose concentration, insulin concentration, and hemoglobin A1C %. High-sensitivity C-reactive protein concentration (hsCRP) was also measured.

The laboratory studies were performed in the institution's certified central laboratory. The lipid panel was measured on a Roche Cobas c501 with appropriate reagents. The technique has documented traceability to the National Reference System for Cholesterol by performing a direct comparison with the cholesterol reference method using fresh human specimens, which cover the National Cholesterol Education Program (NCEP) medical decision points. The system has demonstrated the ability to meet the NCEP's performance criteria for accuracy and precision.

Perceived Stress Scale (PSS)

The PSS is one of the most widely accepted measures of stress (Cohen et al. 1983). This validated 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0–56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Score in the low 20s reveal moderate stress levels, while scores approaching 30 are substantial and concerning.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval (Buysse et al. 1989). Nineteen individual items generate seven component scores whose sum yields one global score with a range of 0–21. The psychometric and clinical properties of the PSQI suggest its utility both in clinical practice and research activities. A global score of greater than 5 indicates a poor sleeper. Sleep perturbations can be categorized by scores: 0–5 is a good sleep score; 6–10 shows mild sleep difficulty; 11–15 moderate sleep difficulty; and 16–21 severe sleep difficulty.

Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) is the most widely used tool to estimate the subjective symptom of daytime sleepiness (Johns 1992). Subjects were asked to use a scale of 0–3 to estimate their likelihood of dozing in eight different situations in recent weeks. The individual scores were summed and possible scores range from 0 to 24. Sleepy subjects score 10 or higher and sleepiness can be categorized by scores: 10–14 as mild sleepiness, 15–19 as moderate sleepiness, and 20–24 as severe sleepiness.

Fatigue Scale

The Fatigue Visual Numeric Scale is borrowed from the Stanford Patient Education Research Center (see http://patienteducation.stanford.edu/research/vnsfatigue.html, accessed 1 July 2010). This fatigue scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. Subjects who circle 5–6 express mild fatigue, 7–8 moderate fatigue, and 9–10 severe fatigue.

Berlin Questionnaire

Of questionnaires available to screen patients for sleep apnea, the Berlin Questionnaire is one of the most commonly utilized and best validated (Netzer et al. 1999). As measured by the questionnaire, patients with persistent and frequent symptoms are considered to be at high risk for sleep apnea. Questions about symptoms demonstrated internal consistency (Cronbach correlations, 0.86–0.92). With a positive Berlin Questionnaire, sleep apnea was predicted with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.89, and a likelihood ratio of 3.79.

Statistical analysis

Data are presented as mean ± SD. Two sample *t*-tests were used to compare continuous variables between groups, and categorical data were compared between groups using Fisher's exact test. Body habitus, sleep variables, and hsCRP did not satisfy assumptions of normality (as tested by the Shapiro–Wilk statistic) therefore, Spearman's correlation coefficient; (r_s) was used to examine the association of these variables with the PSS. All tests were two-tailed and p values < 0.05 were presumed to represent statistical

Table I. Characteristics of the subjects according to perceived stress levels.

	All subjects ($n = 350$)	Low stress ($n = 185$)	High stress ($n = 165$)	t statistic	Degrees of freedom	p value
Age, years (mean ± SD)	54.4 ± 12.4	57.4 ± 11.5	51.1 ± 12.6	4.9	348	<0.001*
Race (%)						
Caucasian	134 (38%)	73 (39%)	60 (36%)			0.673†
African American	105 (30%)	53 (29%)	52 (31%)			
Hispanic	14 (4%)	5 (3%)	9 (5%)			
Asian	2 (1%)	1 (1%)	1 (1%)			
Others	96 (27%)	53 (29%)	43 (26%)			
Gender, male (% male)	138 (39%)	78 (42%)	60 (36%)			0.28†
PSS score (56 points max)	22.4 ± 8.1	16.3 ± 4.7	29.3 ± 5.0	−25.0	348	<0.001*

Notes: Values are mean ± SD or actual number of subjects in a category (with proportion). Statistical comparisons are between low-stress and high-stress subjects using the two sample t-test (or Fisher exact test as noted) with p values less than 0.05 representing statistical significance. Low-stress subjects were defined by a Perceived Stress Score less than the mean of 23 points, while high-stress subjects were defined by a score equal to or greater than the mean of 23 points; * denotes two sample t-test between low-stress and high-stress subjects; † denotes Fisher's exact test between low-stress and high-stress subjects.

significance. Data were analyzed using SPSS for Windows (v. 17.0, SPSS, Inc., (IBM), Chicago, IL, USA).

Results

We studied data from 350 participants entering ICHP's CVD prevention program. The mean age (±SD) of our participants was 54.4 ± 12.4 years, consistent with a spectrum of lifestyles from actively working to semi-retired and fully retired adults. Heavily represented racial categories were Caucasian and African American, but a substantial number of subjects identified themselves as mixed race or declined to pick a category. There was a majority of women (61%) in our study sample (see Table I).

As the mean PSS score was 22.4 points, we elected to define subjects with PSS scores of 23 or more points as belonging to the "high-stress" group and subjects with PSS less than 23 as the "low-stress" group. This allowed for analysis of data for nearly equal sized cohorts of high- and low-stress groups. While there are no defined ranges of "normality" or published degrees of severity based upon the PSS scores, the cut point of 23 does conform to a threshold value above which stress becomes a concerning issue from a clinical point of view in our experience within our program.

As summarized in Table I, there were no significant differences with regard to race or gender for high-stress and low-stress groups, though high-stress subjects were somewhat younger ($p < 0.001$).

As summarized in Table II, the cohort of subjects with high stress had a higher BMI (obese indices vs. merely overweight, $p = 0.001$) and larger measured waist circumferences. The biochemical measurement of hsCRP showed a positive correlation with perceived stress. The high-stress group also showed shorter total sleep times (20 min less per 24 h), poorer sleep quality, higher likelihood of sleep apnea diagnosis, greater sleepiness, and greater fatigue. The correlation between perceived stress and sleep quality is illustrated in Figure 1.

Several measurements ($n = 350$), not presented in the tables, showed no correlation with levels of perceived stress by Spearman's rank correlation. The lipid panel including total serum concentrations of

Table II. Correlations between perceived stress levels vs. anthropometrics, behavior scores, symptom scores, and laboratory values.

	Low stress ($n = 185$)	High stress ($n = 165$)	Correlation coefficient*	p value two-tailed
BMI (kg/m^2)	29.0 ± 5.9	31.1 ± 5.9	0.175	0.0011
Waist circumference (cm)	98 ± 14	102 ± 17	0.135	0.012
Total sleep time (hours/24 h)	6.4 ± 1.2	6.1 ± 1.5	−0.177	0.0011
Pittsburgh Sleep Quality Index (21-point scale)	5.9 ± 4.0	8.5 ± 4.4	0.416	<0.001
Berlin Questionnaire (% high risk for sleep apnea)	63/152 (41)%	72/120 (60%)		0.003†
Epworth Sleepiness Scale (24-point scale)	7.8 ± 4.8	10.4 ± 5.0	0.316	<0.001
Fatigue scale (10-point scale)	3.4 ± 2.4	5.4 ± 2.2	0.484	<0.001
hsCRP (mg/dl)	0.356	0.384	0.109	0.045

Notes: Values are mean + SD or proportion. Statistical comparisons are between low-stress and high-stress subjects using the two sample t-test (or Fisher exact test as noted) with p values less than 0.05 representing statistical significance. Correlation coefficients are derived from the Spearman's rank correlation coefficient (also called Spearman's rho) using a two-tailed test with $p < 0.05$ as the predetermined threshold of statistical significance. BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; * denotes Spearman's rho (r_s); † denotes Fisher's exact test.

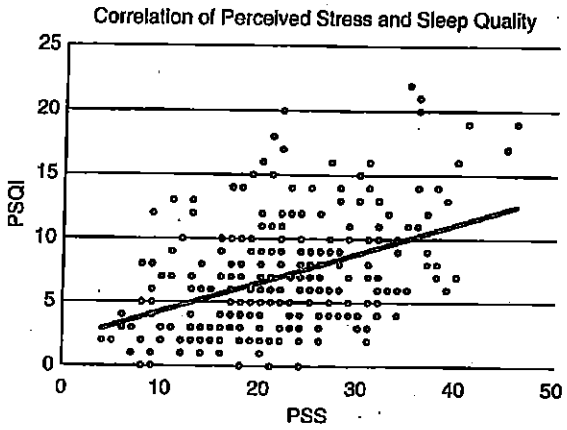

Figure 1. Using the Spearman correlation, there is a significant positive relationship between perceived stress scores (PSS) and scores on the PSQI, $r_s = 0.43$, $n = 274$, $p < 0.0005$.

cholesterol ($r_s = 0.04$), LDL cholesterol ($r_s = 0.004$), HDL cholesterol ($r_s = 0.015$), triglyceride ($r_s = -0.045$), and Lp (a) ($r_s = -0.013$) did not correlate with PSS. Likewise, parameters of glucose metabolism did not correlate with PSS, including fasting plasma glucose concentration ($r_s = 0.057$), HgA1C percentage ($r_s = 0.013$), and the homeostatic assessment model or HOMA ($r_s = 0.093$).

When sorted by gender, important differences were revealed. By *t*-tests ($n = 350$), women were slightly younger (54 ± 11 years vs. 58 ± 12 years, $F = 1.63$, df = 348, $p = 0.04$), had higher perceived stress scores (PSS = 24 ± 8 vs. 20 ± 8, $F = 0.44$, df = 348, $p = 0.04$), had higher total serum cholesterol concentration (200 ± 37 vs. 176 ± 72 mg/dl, $F = 0.58$, df = 343, $p = 0.009$), and higher serum HDL cholesterol concentration (64 ± 22 vs. 48 ± 12 mg/dl, $F = 15.21$, df = 343, $p < 0.001$).

Discussion

The salient findings of this study are that increased levels of perceived stress were correlated with shortened total sleep time, worse scores for sleep quality, higher likelihood of sleep apnea, and worse daytime symptoms of sleepiness and fatigue. It is important to note that there were no concomitant correlations between perceived stress and lipid abnormalities or measures of glucose metabolism, two common risk factors for heart disease. It is known that normal values for lipids and glucose metabolism do not preclude an increased CVD risk. The finding that glucose and lipids did not correlate with stress in our study places greater weight on the role of sleep disruption in the development of CVD. In combination with numerous prior studies that connect short sleep and disturbed sleep with CVD (Heslop et al. 2002a,b), our correlations provide a mechanistic link to support the observed association between stress and CVD.

It is important to define stress and what is actually being measured with the PSS as it pertains to the current investigation. Because the PSS questions are general and free of content specificity, the instrument assesses subjectively experienced stress independent of an objective external stimulus or situation (Cohen et al. 1983). Personality aspects and resources of the subjects contribute to the total perceived stress score. The PSS correlates closely with trait neuroticism rather than the state of stress imposed. It therefore follows that trait neuroticism may be a pre-morbid characteristic of some good sleepers, who nonetheless manifest hyperarousal in response to stress and thus develop stress-induced insomnia (Basta et al. 2007; Fernandez-Mendoza et al. 2010).

The tools used to measure sleep in this study evaluate both sleep quality and sleep quantity. The high-stress group got an average of 20 min less sleep per night compared to the low-stress group. This may initially appear to be an inconsequential difference in sleep quantity. However, after only a few days or weeks, a substantial sleep debt can accrue, sufficient to affect mood, performance, and sense of well-being (Dinges et al. 1997; Drake et al. 2001). Furthermore, fatigue-inducing pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor alpha) are negatively influenced by the quantity and quality of sleep (Vgontzas et al. 1999). CVD is a disease state stimulated and exacerbated by systemic inflammation. Prior research has also shown that insomnia with objective short sleep duration is associated with a higher risk for hypertension (Vgontzas et al. 2009a,b) and for type 2 diabetes mellitus (Vgontzas et al. 2009a,b), both major risk factors for CVD.

The Berlin Questionnaire focuses on an aspect of sleep quality. It is a validated instrument to quantify high vs. low risk for sleep apnea. The high-stress group with substantially higher BMI also has much higher odds of having sleep apnea. This finding is consistent with prior research that correlates increasing BMI with higher risk for sleep apnea (Newman et al. 2005). Explanations of these associations may include alternative theoretical models. Stress may stimulate maladaptive eating, leading to weight gain and subsequent development of sleep apnea. Alternatively, sleep apnea may disrupt the restorative functions of sleep (experienced as higher stress levels) and simultaneously disrupt hormonal regulation of hunger leading to greater calorie consumption and weight gain. These pathways toward greater risk of CVD warrant corrective attention at a time early in the cycle to preclude a downward spiral of health indicators.

Worse sleep quality as measured by the PSQI correlated with higher stress levels (Figure 1). Similarly, the ESS and fatigue scale, consequences

of the impact of poor sleep quality, correlated with higher stress levels. The finding that different tools showed worse scores with higher stress levels gives credibility to the observation linking poor sleep quality with high stress. Of course the challenge will be finding effective ways to improve sleep quality and consequent daytime symptoms, translating to improvements in CVD risk.

A novel aspect of our research is the use of the PSS and PSQI as tools to measure stress and sleep quality. There are few other studies that link perceived stress with poor sleep quality. There is one publication that utilized both the PSS and the PSQI in the same study (Strange et al. 2009). These coauthors investigated 220 pregnant women and found that PSS did not predict preterm birth and that preterm births were associated with lower daytime dysfunction scores on the PSQI. A PSS–PSQI connection was not reported in the study.

CVD is the leading cause of death in women, despite the cardiovascular protection afforded by their endogenous hormones and increased levels of HDL cholesterol (Wasserthiel-Smoller 2010). In our study, women were found to have significantly higher perceived stress scores than men. This finding may indicate that stress levels, specifically the measured PSS score, may be an important gender-relevant risk factor to survey, especially as a preventive strategy for improving women's health.

What cannot be determined in a cross-sectional study is causality. It cannot be inferred whether or not perceived stress causes deranged sleep or if poor sleep habits cause increases in perceived stress. It is possible that both perceived stress and sleep habits are worsened by another stimulus and that they respond in parallel to that stimulus. The relationship of perceived stress and disturbed sleep deserves further clarification, perhaps with a study providing an intervention aimed at stress or at sleep alone.

One limitation of the current study is that several indices were measured using subjective self-reports. Self-reported data included perceived stress levels, sleep quality, daytime sleepiness, and fatigue. However, the tools utilized to gather these indices were validated instruments with known performance characteristics and some of the data sought have no alternative ways of being measured. It may be useful in future studies to utilize objective measures such as a polysomnogram instead of the Berlin Questionnaire for sleep apnea and actigraphy as an objective measure of sleep quantity. Furthermore, strength of the current study is that actual measurements of height, weight, and waist circumference were used in place of self-reported values.

Our finding of correlation of perceived stress levels with sleep disruption adds to the growing body of evidence that stress may play an important role as a risk factor for CVD. Certainly the evidence to date is worthy of follow-up studies. A justifiable next study could examine the impact of stress management strategies and sleep improvement on incident CVD. Assessing maladaptive behaviors and physiological abnormalities associated with stress may allow for targeted intervention to promote vascular health.

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